Baskar, P.
09/670096

09/670355 ; 670096 ; 669843 ; 669833 ; 670244

- key terms

FILE 'CAPLUS' ENTERED AT 10:59:42 ON 14 NOV 2001
L1 42 S (SARCOCYST? OR S)(W)NEURONA
L2 17 S SARCOSPORID?
L3 36 S (L1 OR L2) AND (EQUINE OR EQUID## OR HORSE)
L4 9 S L3 AND (MOAB OR MAB OR ANTIBOD?)

L4 ANSWER 1 OF 9 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2001:810574 CAPLUS
TITLE: Prevalence of agglutinating **antibodies** to **Sarcocystis neurona** in raccoons, Procyon lotor, from the United States
AUTHOR(S): Lindsay, David S.; Rosypal, Alexa C.; Spencer, Jennifer A.; Cheadle, M. Andy; Zajac, Anne M.; Rupprecht, Charles; Dubey, J. P.; Blagburn, Byron L.
CORPORATE SOURCE: 1410 Prices Fork Road, Virginia Tech, Center for Molecular Medicine and Infectious Diseases, Department of Biomedical Sciences and Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine, 24061-0342, Blacksburg, VA, USA
SOURCE: Vet. Parasitol. (2001), 100(3-4), 131-134
CODEN: VPARDI; ISSN: 0304-4017
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Equine** protozoal myeloencephalitis (EPM) is the most important protozoal disease of **horses** in North America and it is caused by **Sarcocystis neurona**. Natural cases of encephalitis due to **S. neurona** have been reported in raccoons, Procyon lotor. We examd. 99 raccoons for agglutinating **antibodies** to **S. neurona** using the **S. neurona** agglutination test (SAT) employing formalin-fixed merozoites as antigen. Raccoons originated in Florida (N=24, collected in 1996), New Jersey (N=25, collected in 1993), Pennsylvania (N=25, collected in 1999), and Massachusetts (N=25, collected in 1993 and 1994). We found that 58 (58.6%) of the 99 raccoons were pos. for **antibodies** to **S. neurona** using the SAT; 44 of 99 raccoons (44%) had titers of .gtoreq.1:500. This prevalence is similar to the reported seroprevalence of 33-60% for **S. neurona** **antibodies** in **horses** from the United States using the Western blot test.

L4 ANSWER 2 OF 9 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2001:167817 CAPLUS
DOCUMENT NUMBER: 134:221431
TITLE: Vaccine to control **equine** protozoal myeloencephalitis in **horses**
INVENTOR(S): Mansfield, Linda S.; Rossano, Mary G.; Murphy, Alice J.; Vrable, Ruth A.
PATENT ASSIGNEE(S): Michigan State University, USA
SOURCE: PCT Int. Appl., 57 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 2001015708 | A1 | 20010308 | WO 2000-US24221 | 20000831 |

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 1999-152193 P 19990902
US 2000-513086 A 20000224

AB The present invention provides vaccines and methods for making the vaccines that actively or passively protect an **equid** or other animal against **Sarcocystis neurona**. In particular, the present invention provides vaccines that provide active immunity which comprise a polypeptide or DNA vaccine that contains or expresses at least one epitope of an antigen that has an amino acid sequence substantially similar to a unique 16 (+/-4) kDa antigen and/or 30 (+/-4) kDa antigen of **Sarcocystis neurona**. The present invention further provides a vaccine that provides passive immunity to **Sarcocystis neurona** comprising polyclonal or monoclonal **antibodies** against at least one epitope of an antigen substantially similar to a unique 16 (+/-4) kDa antigen and/or 30 (+/-4) kDa antigen of **Sarcocystis neurona**.

REFERENCE COUNT: 1
REFERENCE(S): (1) Liang; Infection and Immunity 1998, V66(5), P1834 CAPLUS

L4 ANSWER 3 OF 9 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2001:129329 CAPLUS
TITLE: Direct agglutination test for the detection of **antibodies** to **Sarcocystis neurona** in experimentally infected animals
AUTHOR(S): Lindsay, D. S.; Dubey, J. P.
CORPORATE SOURCE: Center for Molecular Medicine and Infectious Diseases, Department of Biomedical Sciences and Pathobiology, Virginia Tech, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA, 24061-0342, USA
SOURCE: Vet. Parasitol. (2001), 95(2-4), 179-186
CODEN: VPARDI; ISSN: 0304-4017
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Equine** protozoal myeloencephalitis (EPM) is a serious neurol. disease of **horses** in the Americas. The apicomplexan protozoan most commonly assocd. with EPM is **Sarcocystis neurona**. A direct agglutination test (SAT) was developed to detect **antibodies** to **S. neurona** in exptl. infected animals. Merozoites of the SN6 strain of **S. neurona** collected from cell culture were used as antigen and 2-mercaptoethanol was added to the antigen

suspension to destroy IgM **antibodies** when mixed with test sera. Mice fed sporocysts of S. speeri or S. falcatula-like sporocysts from opossums did not seroconvert in the SAT. The sensitivity of the SAT was 100% and the specificity was 90% in mice.

REFERENCE COUNT: 26

REFERENCE(S):
(1) Ardoin, P; C R Soc Biol 1967, V161, P117 MEDLINE
(2) Beech, J; Vet Pathol 1974, V11, P87 MEDLINE
(3) Bentz, B; J Am Vet Med Assoc 1997, V210, P517 MEDLINE
(5) Cusick, P; J Am Vet Med Assoc 1974, V164, P77 MEDLINE
(6) Cutler, T; J Parasitol 1999, V85, P301 MEDLINE

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 4 OF 9 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2001:129327 CAPLUS

DOCUMENT NUMBER: 135:2611

TITLE: Characteristics of a recent isolate of **Sarcocystis neurona** (SN7) from a **horse** and loss of pathogenicity of isolates SN6 and SN7 by passages in cell culture

AUTHOR(S): Dubey, J. P.; Mattson, D. E.; Speer, C. A.; Hamir, A. N.; Lindsay, D. S.; Rosenthal, B. M.; Kwok, O. C. H.; Baker, R. J.; Mulrooney, D. M.; Tornquist, S. J.; Gerros, T. C.

CORPORATE SOURCE: Agricultural Research Service, Animal and Natural Resources Institute, Parasite Biology, Epidemiology and Systematics Laboratory, United States Department of Agriculture, Beltsville Agricultural Research Center, Beltsville, MD, 20705-2350, USA

SOURCE: Vet. Parasitol. (2001), 95(2-4), 155-166
CODEN: VPARDI; ISSN: 0304-4017

PUBLISHER: Elsevier Science B.V.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB An isolate of **Sarcocystis neurona** (SN7) was obtained from the spinal cord of a **horse** with neurol. signs. The parasite was isolated in cultures of bovine monocytes and **equine** spleen cells. The organism divided by endopolygeny and completed at least one asexual cycle in cell cultures in 3 days. The parasite was maintained by subpassages in bovine monocytes for 10 mo when it was found to be non-pathogenic to gamma interferon knockout (KO) mice. Revival of a low passage (10th passage) of the initial isolate stored in liq. nitrogen for 18 mo retained its pathogenicity for KO mice. Merozoites (106) of the late passage (22nd passage) were infective to only one of four KO mice inoculated. Similar results were obtained with SN6 isolate of **S. neurona**. No differences were found in Western blot patterns using antigens from the low and high passage merozoites of the SN7 and SN6 isolates. These results suggest that prolonged passage in cell culture may affect the pathogenicity of some isolates of **S. neurona**.

REFERENCE COUNT: 25

REFERENCE(S):
(14) Liang, F; Infect Immun 1998, V66, P1834 CAPLUS

(17) Lindsay, D; J Parasitol 2000, V86, P164
CAPLUS
(19) Lindsay, D; Vet Parasitol 1999, V82, P205
CAPLUS
(21) Marsh, A; Am J Vet Res 1996, V57, P975
CAPLUS
(23) Rosenthal, B; Vet Parasitol 2001, V95, P133
CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 5 OF 9 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2001:129326 CAPLUS
DOCUMENT NUMBER: 135:2610
TITLE: Characterization of a **Sarcocystis neurona** isolate from a Missouri **horse** with **equine** protozoal myeloencephalitis
AUTHOR(S): Marsh, A. E.; Johnson, P. J.; Ramos-Vara, J.; Johnson, G. C.
CORPORATE SOURCE: College of Veterinary Medicine, Department of Veterinary Pathobiology, University of Missouri, Columbia, MO, 65211, USA
SOURCE: Vet. Parasitol. (2001), 95(2-4), 143-154
CODEN: VPARDI; ISSN: 0304-4017
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Little information is available about antigenic variation of **Sarcocystis neurona** isolated from **horses** with **equine** protozoal myeloencephalitis, nor is there much information available on the specific **antibody** pattern to **S. neurona** antigens of **horses** from different geog. regions where **S. neurona** isolates have been obtained. This communication reports on the characterization of a new **S. neurona** isolate, SN-MU1. The isolate was obtained from a 3-yr old Thoroughbred that had asym. neurol. signs and localized skeletal muscle atrophy. This **S. neurona** isolate is similar to other **S. neurona** isolates by mol. anal. of the internal transcribed spacer (ITS-1) region and a random-amplified polymorphic DNA marker, but is phenotypically distinct from the other **S. neurona** isolates examd. Evaluation of the **antibodies** from the affected **horse** and immunohistochem. results suggested that antigenic variation of **S. neurona** can result in variable **antibody**-antigen reactivity obsd. in the **S. neurona** immunoblot test.
REFERENCE COUNT: 40
REFERENCE(S): (23) Liang, F; Anal Biochem 1997, V250, P61
CAPLUS
(24) Liang, F; Infect Immun 1998, V66, P1834
CAPLUS
(26) Marsh, A; Am J Vet Res 1996, V57, P975
CAPLUS
(29) Marsh, A; J Parasitol 1999, V85, P750
CAPLUS
(39) Tanhauser, S; J Parasitol 1999, V85, P221
CAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 6 OF 9 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2000:592749 CAPLUS
DOCUMENT NUMBER: 133:191998
TITLE: An antigen test to detect **equine** protozoal myeloencephalitis in **horse** serum and cerebrospinal fluid
INVENTOR(S): Mansfield, Linda S.; Rossano, Mary G.; Murphy, Alice J.; Vrable, Ruth A.
PATENT ASSIGNEE(S): Michigan State University, USA
SOURCE: PCT Int. Appl., 64 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 2000049049 | A1 | 20000824 | WO 2000-US4379 | 20000218 |

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 1999-120831 P 19990219
US 1999-152193 P 19990902

AB The present invention provides an immunoassay to detect identifying antigens in **horses** that are infected with **Sarcocystis neurona** . The immunoassay is preferably an antigen-capture-based assay that relies upon polyclonal or monoclonal **antibodies** against a 16 (<u4) and/or 30 (<u4) kDa antigens specific to **Sarcocystis neurona** to detect the presence of the 16 (<u4) and/or 30 (<u4) kDa antigens in **equine** serum or **equine** cerebrospinal fluid.

REFERENCE COUNT: 3
REFERENCE(S):
(1) Catty; Antibodies Volume II a practical approach 1989, P97
(2) Goding, J; Moloclonal Antibodies:Principles and Practice London 1983, P56
(3) Liang; Infection and Immunity 1998, V66(5), P1834 CAPLUS

L4 ANSWER 7 OF 9 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2000:210497 CAPLUS
DOCUMENT NUMBER: 132:250014
TITLE: Immunoassay for **equine** protozoal myeloencephalitis in **horses**
INVENTOR(S): Mansfield, Linda S.; Murphy, Alice J.; Rossano, Mary G.
PATENT ASSIGNEE(S): Michigan State University, USA
SOURCE: PCT Int. Appl., 26 pp.

CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 2000017640 | A1 | 20000330 | WO 1999-US17961 | 19990809 |
| W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | | |
| RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG | | | | |
| US 6153394 | A | 20001128 | US 1998-156954 | 19980918 |
| AU 9954707 | A1 | 20000410 | AU 1999-54707 | 19990809 |

PRIORITY APPLN. INFO.: US 1998-156954 A 19980918
WO 1999-US17961 W 19990809

AB An immunoassay for **Sarcocystis neurona** **antibodies** in **equines** is described. The immunoassay uses blocking of Sarcocystis antigens by **antibodies** to Sarcocystis sp. other than **Sarcocystis neurona** in connection with the immunoassay.

REFERENCE COUNT: 4
REFERENCE(S):
(1) Boyer; US 5399484 A 1995 CAPLUS
(2) Granstrom; Journal Vet Diagn Invest 1993, V5, P88 MEDLINE
(3) Marsh; JAVMA 1996, V209(11), P1907 MEDLINE
(4) Murthy; Clin Chem 1986, V32(10), P1956 CAPLUS

L4 ANSWER 8 OF 9 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:809562 CAPLUS
DOCUMENT NUMBER: 132:277922
TITLE: Prevalence of **antibodies** to Neospora caninum in dogs
AUTHOR(S): Cheadle, M. A.; Lindsay, D. S.; Rowe, S.; Dykstra, C. C.; Williams, M. A.; Spencer, J. A.; Toivio-Kinnucan, M. A.; Lenz, S. D.; Newton, J. C.; Rolsma, M. D.; Blagburn, B. L.
CORPORATE SOURCE: Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, AL, 36849, USA
SOURCE: Int. J. Parasitol. (1999), 29(10), 1537-1543
CODEN: IJPYBT; ISSN: 0020-7519
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB An IFAT was used to det. the prevalence of Neospora-specific IgG **antibodies** in serum from Alabama **horses**. Serum samples (n = 536) were from asymptomatic **horses** routinely submitted for **equine** infectious anemia virus infection testing. We also subjected a 13-yr-old **horse** with CNS disease to necropsy examn. for isolation and in vitro cultivation of

protozoal organisms. In antemortem tests, this **horse** was pos. for **antibodies** to Neospora sp. in the IFAT and western immunoblot. Results of the prevalence survey indicated that IgG **antibodies** to Neospora were present in 62 (11.5%) of the 536 serum samples. Endpoint titers for the pos. samples were 1:50 (35/6.5%), 1:100 (19/3.5%), 1:200 (7/1.3%) and 1:1600 (1/0.2%). Tachyzoites were first seen in cultured bovine turbinate cells 32 days after inoculation with spinal cord homogenates from the **horse** with CNS disease. Tachyzoites reacted with known N. caninum-pos. serum from **horses**, cows, dogs and mice, but did not react with murine anti-Toxoplasma gondii or **equine** anti-**Sarcocystis neurona** serum. Ultrastructural features of tachyzoites and results of comparison of tachyzoite immunodominant proteins revealed that they were identical to those of N. hughesi, a species described recently from a naturally infected **horse**. The isolate recovered from the naturally infected **horse** in the present study (designated NA1) is thought to be an isolate of N. hughesi, although confirmation of this awaits addnl. mol. characterization. These results provide some addnl. evidence that N. hughesi is a valid species and that Neospora infections in **horses** may occur in widely sepd. geog. regions of the United States.

REFERENCE COUNT: 25
REFERENCE(S):
(1) Barr, B; J Vet Diagn Invest 1991, V3, P39
MEDLINE
(14) Howe, D; Infect Immun 1998, V66, P5322
CAPLUS
(16) Lindsay, D; Am J Vet Res 1994, V55, P976
CAPLUS
(19) Marsh, A; Int J Parasitol 1999, V29, P1575
CAPLUS
(21) Marsh, A; J Parasitol 1995, V81, P530
CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 9 OF 9 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1998:296904 CAPLUS
DOCUMENT NUMBER: 129:39929
TITLE: Evidence that surface proteins Sn14 and Sn16 of **Sarcocystis neurona** merozoites are involved in infection and immunity
AUTHOR(S): Liang, Fang Ting; Granstrom, David E.; Zhao, Xiao Min; Timoney, John F.
CORPORATE SOURCE: Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, Lexington, KY, 40546, USA
SOURCE: Infect. Immun. (1998), 66(5), 1834-1838
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Sarcocystis neurona** is the etiol. agent of **equine** protozoal myeloencephalitis (EPM). Based on an anal. of 25,000 **equine** serum and cerebrospinal fluid (CSF) samples, including samples from **horses** with neurol. signs typical of EPM or with histol. or parasitol. confirmed EPM, four major immunoblot band patterns have been identified. Twenty-three serum and CSF samples representing each of the four immunoblot

patterns were selected from 220 samples from **horses** with neurol. signs resembling EPM and examd. for inhibitory effects on the infectivity of **S. neurona** by an in vitro neutralization assay. A high correlation between immunoblot band pattern and neutralizing activity was detected. Two proteins, Sn14 and Sn16 (14 and 16 kDa, resp.), appeared to be important for in vitro infection. A combination of the results of surface protein labeling, immunopptn., Western blotting, and trypsin digestion suggests that these mols. are surface proteins and may be useful components of a vaccine against **S. neurona** infection. Although **S. neurona** is an obligate intracellular parasite, it is potentially a target for specific **antibodies** which may lyse merozoites via complement or inhibit their attachment and penetration to host cells.

(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, JAPIO, CABA, AGRICOLA, VETU, VETB' ENTERED AT 11:26:47 ON 14 NOV 2001)

L5 236 S L4
L6 50 S L5 AND ANTIGEN
L7 23 DUP REM L6 (27 DUPLICATES REMOVED)

L7 ANSWER 1 OF 23 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 2001-218486 [22] WPIDS
CROSS REFERENCE: 2000-571969 [49]
DOC. NO. CPI: C2001-065294
TITLE: Vaccinating **equids** against protozoal **Sarcocystis neurona** infections using unique **antigens.**
DERWENT CLASS: B04 C06 D16
INVENTOR(S): MANSFIELD, L S; MURPHY, A J; ROSSANO, M G; VRABLE, R A
PATENT ASSIGNEE(S): (UNMS) UNIV MICHIGAN STATE
COUNTRY COUNT: 87
PATENT INFORMATION:

| PATENT NO | KIND | DATE | WEEK | LA | PG |
|---|---|---|---|---|---|
| WO 2001015708 | A1 | 20010308 | (200122)* | EN | 54 |

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG UZ VN YU ZA ZW

AU 2000071087 A 20010326 (200137)

APPLICATION DETAILS:

| PATENT NO | KIND | APPLICATION | DATE |
|---|---|---|---|
| WO 2001015708 | A1 | WO 2000-US24221 | 20000831 |
| AU 2000071087 | A | AU 2000-71087 | 20000831 |

FILING DETAILS:

| PATENT NO | KIND | PATENT NO |
|---|---|---|

AU 2000071087 A Based on WO 200115708

PRIORITY APPLN. INFO: US 2000-513086 20000224; US 1999-152193 19990902

AN 2001-218486 [22] WPIDS

CR 2000-571969 [49]

AB WO 200115708 A UPAB: 20010704

NOVELTY - Vaccinating **equids** against **Sarcocystis neurona** infections using polypeptide groups of unique 16 (+4) or 30 (+4) **antigens** of **S. neurona**, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following:

(a) a vaccine (I) for providing passive immunity to **Sarcocystis neurona** infection, comprising **antibodies** against at least one group of a unique 16 (+4) or 30 (+4) **antigen** of **S. neurona**;

(b) a vaccine (II) for active immunization of an **equid** against a **S. neurona** infection, comprising at least one group of a unique 16 (+4) or 30 (+4) **antigen** of **S. neurona**;

(c) a vaccine (III) for protecting an **equid** from **S. neurona** infection comprising a DNA that encodes at least 1 group of a 16 (+4) kDa **antigen** and/or a 30 (+4) kDa **antigen** of **S. neurona**;

(d) a method (IV) for vaccinating an **equid** against a **S. neurona** infection, comprising:

(1) providing a recombinant **antigen** of **S. neurona** produced from a recombinant microorganism culture (the microorganism contains a DNA that encodes at least one group of a 16 (+4) kDa **antigen** and/or 30 (+4) kDa **antigen** of **S. neurona**; and

(2) vaccinating the **equid**;

(e) a method (V) for vaccinating an **equid** against a **S. neurona** infection, comprising:

(1) providing a DNA in a carrier solution, a plasmid which encodes at least 1 group of a 16 (+4) kDa **antigen** and/or 30 (+4) kDa **antigen** of **Sarcocystis neurona**; and

(2) vaccinating the **equid** with the DNA in the carrier solution;

(f) a method (VI) of providing passive immunity to a **S. neurona** infection in a **equid**, comprising:

(1) providing **antibodies** against at least 1 group of a 16 (+4) kDa **antigen** and/or 30 (+4) kDa **antigen** of **S. neurona** (the **antibodies** may be monoclonal or polyclonal); and

(2) inoculating the **equid**;

(g) a method (VII) for producing a polypeptide, comprising:

(1) providing a microorganism in a culture containing a DNA encoding a fusion polypeptide comprising at least 1 group of a 16 (+4) kDa **antigen** and/or 30 (+4) kDa **antigen** of **S. neurona** and a polypeptide that facilitates isolation of the fusion polypeptide;

(2) culturing the microorganism in a culture to produce the fusion polypeptide; and

(3) isolating the fusion polypeptide;

(h) a method (VIII) for producing an **antibody**

comprising:
(1) providing a microorganism in a culture containing DNA encoding a fusion polypeptide comprising at least 1 group of a 16 (+4) kDa **antigen** and/or 30 (+4) kDa **antigen** of **S. neurona** and a polypeptide that facilitates isolation of the fusion polypeptide;
(2) culturing the microorganisms in a culture to produce the fusion polypeptide;
(3) isolating the fusion polypeptide;
(4) producing the **antibody** from the polypeptide;
(i) a monoclonal **antibody** (IX) that selectively binds to a 16 (+4) kDa **antigen** and/or 30 (+4) kDa **antigen**;
(j) an isolated DNA (X) encoding a monoclonal **antibody** that selectively binds to a 16 (+4) kDa **antigen** and/or 30 (+4) kDa **antigen**;
(k) a bacterial clone (XI) containing a plasmid comprising a DNA encoding a 16 (+4) kDa **antigen** and/or 30 (+4) kDa **antigen** of **S. neurona**;
(l) a vaccine (XII) for an **equid** comprising an isolated recombinant protein encoded by a cDNA produced from mRNA of **S. neurona** encoding a 16 (+4) kDa **antigen** and/or 30 (+4) kDa **antigen**;
(m) a vaccine (XIII) for an **equid** comprising a recombinant virus vector containing DNA encoding a 16 (+4) kDa **antigen** and/or 30 (+4) kDa **antigen** of **S. neurona**;
(n) a DNA vaccine (XIV) for an **equid** comprising a plasmid containing DNA encoding a 16 (+4) kDa **antigen** and/or 30 (+4) kDa **antigen** of **S. neurona** ; and
(o) a method (XV) for protecting an equid against S. neurona which comprises providing a vaccine that when injected into the equid causes the equid to produce antibodies against a 16 (+4) kDa antigen and/or 30 (+4) kDa antigen of S. neurona (the antibodies prevent infection by the Sarcocystis neurona).
ACTIVITY - Antiparasitic.
No biological data given.
MECHANISM OF ACTION - Vaccine.
USE - The vaccines and methods are used for protecting equids against infections by the protozoan parasite Sarcocystis neurona.
Dwg.0/0

L7 ANSWER 2 OF 23 AGRICOLA
ACCESSION NUMBER: 2001:52514 AGRICOLA
DOCUMENT NUMBER: IND23214214
TITLE: The nine-banded armadillo (Dasypus novemcinctus) is naturally infected with **Sarcocystis neurona**.
AUTHOR(S): Tanhauser, S.M.; Cheadle, M.A.; Massey, E.T.; Mayer, B.A.; Schroedter, D.E.; Dame, J.B.; Greiner, E.C.; MacKay, R.J.
AVAILABILITY: DNAL (QH547.I55)
SOURCE: International journal for parasitology, Apr 2001. Vol. 31, No. 4, p. 325-329
Publisher: Oxford : Elsevier Science Ltd.
CODEN: IJPYBT; ISSN: 0020-7519
NOTE: Includes references

PUB. COUNTRY: England; United Kingdom
DOCUMENT TYPE: Article
FILE SEGMENT: Non-U.S. Imprint other than FAO
LANGUAGE: English

AB Sarcocysts were dissected from the tongue of a nine-banded armadillo (Dasypus novemcinctus). DNA was extracted and characterised by PCR amplification followed by restriction fragment length polymorphism analysis and nucleotide sequencing. A total of 1879 nucleotides were compared; the sarcocyst DNA sequence was identical to that reported for **Sarcocystis neurona** . DNA was extracted from the sarcocysts of five more nine-banded armadillos. A 254-nucleotide sequence was determined for each and found to be identical to **S. neurona**. Western blot techniques for detection of anti-**S. neurona** **antibody** were developed for use with armadillo plasma and samples from 19 wild-caught and 17 captive-raised armadillos were examined. Whereas all of the 19 wild-caught armadillos had **antibodies** to **S. neurona**, only one of 17 captive-raised armadillos did. These results suggest that the nine-banded armadillo are naturally infected with **S. neurona**.

L7 ANSWER 3 OF 23 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 2001354025 MEDLINE
DOCUMENT NUMBER: 21127325 PubMed ID: 11223207
TITLE: Prevalence of Neospora hughesi and **Sarcocystis neurona** **antibodies** in **horses** from various geographical locations.
AUTHOR: Vardeleon D; Marsh A E; Thorne J G; Loch W; Young R; Johnson P J
CORPORATE SOURCE: Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Missouri, Columbia 65211, USA.
SOURCE: VETERINARY PARASITOLOGY, (2001 Feb 26) 95 (2-4) 273-82.
Journal code: XBU; 7602745. ISSN: 0304-4017.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200106
ENTRY DATE: Entered STN: 20010625
Last Updated on STN: 20010625
Entered Medline: 20010621

AB Parasite-specific **antibody** responses to Neospora **antigens** were detected using the immunofluorescent **antibody** test (IFAT) and immunoblot analysis in select **equine** populations. For comparison, a naturally infected Neospora hughesi **horse** and an experimentally inoculated Neospora caninum **horse** were used. In addition, all samples were tested for **antibodies** to **Sarcocystis neurona** by immunoblot analysis. A total of 208 samples was evaluated. The **equine** populations were derived from five distinct geographic regions. Locations were selected based on distribution of Didelphis virginiana, the native North American opossum which serves as the definitive host for **S. neurona**. Only 11% of the samples that had positive titers of

1:100 using the IFAT were also positive for **antibodies** by immunoblot analysis in this study. Overall, there was a 2% seroprevalence for Neospora **antibodies** in all **horses** tested based on immunoblot analysis described. The seroprevalence for **S. neurona antibodies** varied from 0% (New Zealand and Montana) to 54% (Missouri). We concluded that, in testing for **antibodies** against Neospora **antigens** using either IFAT or immunoblot analysis, as described, positive results should not be attributed to the presence of **antibodies** to **S. neurona**.

L7 ANSWER 4 OF 23 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 2001354016 MEDLINE
DOCUMENT NUMBER: 21127316 PubMed ID: 11223198
TITLE: Direct agglutination test for the detection of **antibodies** to **Sarcocystis neurona** in experimentally infected animals.
AUTHOR: Lindsay D S; Dubey J P
CORPORATE SOURCE: Department of Biomedical Sciences and Pathobiology, Center for Molecular Medicine and Infectious Diseases, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg 24061-0342, USA.. lindsayd@vt.edu
SOURCE: VETERINARY PARASITOLOGY, (2001 Feb 26) 95 (2-4) 179-86.
Journal code: XBU; 7602745. ISSN: 0304-4017.
PUB. COUNTRY: Netherlands
(EVALUATION STUDIES)
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200106
ENTRY DATE: Entered STN: 20010625
Last Updated on STN: 20010625
Entered Medline: 20010621

AB **Equine** protozoal myeloencephalitis (EPM) is a serious neurological disease of **horses** in the Americas. The apicomplexan protozoan most commonly associated with EPM is **Sarcocystis neurona**. A direct agglutination test (SAT) was developed to detect **antibodies** to **S. neurona** in experimentally infected animals. Merozoites of the SN6 strain of **S. neurona** collected from cell culture were used as **antigen** and 2-mercaptoethanol was added to the **antigen** suspension to destroy IgM **antibodies** when mixed with test sera. Mice fed sporocysts of S. speeri or S. falcatula-like sporocysts from opossums did not seroconvert in the SAT. The sensitivity of the SAT was 100% and the specificity was 90% in mice.

L7 ANSWER 5 OF 23 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 2001354014 MEDLINE
DOCUMENT NUMBER: 21127314 PubMed ID: 11223196
TITLE: Characteristics of a recent isolate of **Sarcocystis neurona** (SN7) from a **horse** and loss of pathogenicity of isolates SN6 and SN7 by passages in cell culture.
AUTHOR: Dubey J P; Mattson D E; Speer C A; Hamir A N; Lindsay D S; Rosenthal B M; Kwok O C; Baker R J; Mulrooney D

M; Tornquist S J; Gerros T C
CORPORATE SOURCE: United States Department of Agriculture, Agricultural Research Service, Animal and Natural Resources Institute, Beltsville Agricultural Research Center, MD 20705-2350, USA.. jdubey@anri.barc.usda.gov
SOURCE: VETERINARY PARASITOLOGY, (2001 Feb 26) 95 (2-4) 155-66.
Journal code: XBU; 7602745. ISSN: 0304-4017.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200106
ENTRY DATE: Entered STN: 20010625
Last Updated on STN: 20010625
Entered Medline: 20010621

AB An isolate of **Sarcocystis neurona** (SN7) was obtained from the spinal cord of a **horse** with neurologic signs. The parasite was isolated in cultures of bovine monocytes and **equine** spleen cells. The organism divided by endopolygeny and completed at least one asexual cycle in cell cultures in 3 days. The parasite was maintained by subpassages in bovine monocytes for 10 months when it was found to be non-pathogenic to gamma interferon knockout (KO) mice. Revival of a low passage (10th passage) of the initial isolate stored in liquid nitrogen for 18 months retained its pathogenicity for KO mice. Merozoites (10(6)) of the late passage (22nd passage) were infective to only one of four KO mice inoculated. Similar results were obtained with SN6 isolate of **S. neurona**. No differences were found in Western blot patterns using **antigens** from the low and high passage merozoites of the SN7 and SN6 isolates. These results suggest that prolonged passage in cell culture may affect the pathogenicity of some isolates of **S. neurona**.

L7 ANSWER 6 OF 23 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 2001354013 MEDLINE
DOCUMENT NUMBER: 21127313 PubMed ID: 11223195
TITLE: Characterization of a **Sarcocystis neurona** isolate from a Missouri **horse** with **equine** protozoal myeloencephalitis.
AUTHOR: Marsh A E; Johnson P J; Ramos-Vara J; Johnson G C
CORPORATE SOURCE: Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Missouri, Connaway Hall, 1600 East Rollins Dr., Columbia, MO 65211, USA.. marshae@missouri.edu
SOURCE: VETERINARY PARASITOLOGY, (2001 Feb 26) 95 (2-4) 143-54.
Journal code: XBU; 7602745. ISSN: 0304-4017.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200106
ENTRY DATE: Entered STN: 20010625
Last Updated on STN: 20010625
Entered Medline: 20010621

AB Little information is available about antigenic variation of **Sarcocystis neurona** isolated from **horses**

with **equine** protozoal myeloencephalitis, nor is there much information available on the specific **antibody** pattern to **S. neurona antigens** of **horses** from different geographic regions where **S. neurona** isolates have been obtained. This communication reports on the characterization of a new **S. neurona** isolate, SN-MU1. The isolate was obtained from a 3-year old Thoroughbred that had asymmetrical neurological signs and localized skeletal muscle atrophy. This **S. neurona** isolate is similar to other **S. neurona** isolates by molecular analysis of the internal transcribed spacer (ITS-1) region and a random-amplified polymorphic DNA marker, but is phenotypically distinct from the other **S. neurona** isolates examined. Evaluation of the **antibodies** from the affected **horse** and immunohistochemical results suggested that antigenic variation of **S. neurona** can result in variable **antibody-antigen** reactivity observed in the **S. neurona** immunoblot test.

L7 ANSWER 7 OF 23 MEDLINE
ACCESSION NUMBER: 2001646588 IN-PROCESS
DOCUMENT NUMBER: 21555861 PubMed ID: 11698158
TITLE: Prevalence of agglutinating **antibodies** to **Sarcocystis neurona** in raccoons, Procyon lotor, from the United States.
AUTHOR: Lindsay D S; Rosypal A C; Spencer J A; Cheadle M A; Zajac A M; Rupprecht C; Dubey J P; Blagburn B L
CORPORATE SOURCE: Department of Biomedical Sciences and Pathobiology, Center for Molecular Medicine and Infectious Diseases, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, 1410 Prices Fork Road, 24061-0342, Blacksburg, VA, USA.
SOURCE: VETERINARY PARASITOLOGY, (2001 Oct 24) 100 (3-4) 131-4.
Journal code: XBU; 7602745. ISSN: 0304-4017.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: IN-PROCESS; NONINDEXED; Priority Journals
ENTRY DATE: Entered STN: 20011108
Last Updated on STN: 20011108

AB **Equine** protozoal myeloencephalitis (EPM) is the most important protozoal disease of **horses** in North America and it is caused by **Sarcocystis neurona**. Natural cases of encephalitis due to **S. neurona** have been reported in raccoons, Procyon lotor. We examined 99 raccoons for agglutinating **antibodies** to **S. neurona** using the **S. neurona** agglutination test (SAT) employing formalin-fixed merozoites as **antigen**. Raccoons originated in Florida (N=24, collected in 1996), New Jersey (N=25, collected in 1993), Pennsylvania (N=25, collected in 1999), and Massachusetts (N=25, collected in 1993 and 1994). We found that 58 (58.6%) of the 99 raccoons were positive for **antibodies** to **S. neurona** using the SAT; 44 of 99 raccoons (44%) had titers of >/=1:500. This prevalence is similar to the reported seroprevalence of 33-60% for **S. neurona antibodies** in **horses** from the United States using the Western blot test.

L7 ANSWER 8 OF 23 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 2001:258224 BIOSIS
DOCUMENT NUMBER: PREV200100258224
TITLE: Immunoassay for **equine** protozoal myeloencephalitis in **horses**.
AUTHOR(S): Mansfield, Linda S. (1); Murphy, Alice J.; Rossano, Mary G.
CORPORATE SOURCE: (1) Bath, MI USA
ASSIGNEE: Board of Trustees operating Michigan State University
PATENT INFORMATION: US 6153394 November 28, 2000
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Nov. 28, 2000) Vol. 1240, No. 4, pp. No Pagination. e-file.
ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
AB An immunoassay for **Sarcocystis neurona** **antibodies** in **equines** is described. The immunoassay uses blocking of Sarcocystis **antigens** by **antibodies** to Sarcocystis sp. other than **Sarcocystis neurona** in connection with the immunoassay.

L7 ANSWER 9 OF 23 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 2000-571969 [53] WPIDS
CROSS REFERENCE: 2001-218486 [22]
DOC. NO. NON-CPI: N2000-423167
DOC. NO. CPI: C2000-170452
TITLE: Detection of **Sarcocystis neurona** , which causes **equine** protozoal myeloencephalitis, in **horse** serum and cerebrospinal fluid comprises identifying a specific **antibody-antigen** complex via an immunoassay.
DERWENT CLASS: B04 C07 D16 S03
INVENTOR(S): MANSFIELD, L S; MURPHY, A J; ROSSANO, M G; VRABLE, R A
PATENT ASSIGNEE(S): (UNMS) UNIV MICHIGAN STATE
COUNTRY COUNT: 86
PATENT INFORMATION:

| PATENT NO | KIND | DATE | WEEK | LA | PG |
|---|---|---|---|---|---|
| WO 2000049049 | A1 | 20000824 | (200053)* | EN | 64 |
| RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW | | | | | |
| W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG UZ VN YU ZA ZW | | | | | |
| AU 2000034982 | A | 20000904 | (200103) | | |

APPLICATION DETAILS:

| PATENT NO | KIND | APPLICATION | DATE |
|---|---|---|---|
| WO 2000049049 | A1 | WO 2000-US4379 | 20000218 |

09/670355 ; 670096 ; 669843 ; 669833 ; 670244

AU 2000034982 A AU 2000-34982 20000218

FILING DETAILS:

| PATENT NO | KIND | PATENT NO |
|---|---|---|
| AU 2000034982 A | Based on | WO 200049049 |

PRIORITY APPLN. INFO: US 1999-152193 19990902; US 1999-120831 19990219

AN 2000-571969 [53] WPIDS
CR 2001-218486 [22]
AB WO 200049049 A UPAB: 20010421
NOVELTY - Detection of **Sarcocystis neurona** in **horses** by identifying a specific **antibody-antigen** complex via an immunoassay is new.

DETAILED DESCRIPTION - Detection of **Sarcocystis neurona** in an **equine** in an immunoassay is improved by reacting a biological sample from the **horse** suspected of harboring the **S. neurona** with an **antibody** (Ab) which is selective in binding to an identifying **S. neurona antigen** (Ag) to form an Ab-Ag complex.

INDEPENDENT CLAIMS are also included for the following:

(1) a kit for detecting **S. neurona** in a biological sample from an **equine**;

(2) monoclonal **antibodies** against 16 plus or minus 4 kDa or 30 plus or minus 4 kDa **antigens** of **S. neurona**; and

(3) isolated DNA sequences encoding the 16 plus or minus 4 kDa and 30 plus or minus 4 kDa **antigens** of **S. neurona**.

USE - The methods and **antibodies** are useful for detecting **S. neurona** (claimed) which causes **equine** protozoal myeloencephalitis, a neurological disorder in **horses**.

Dwg.0/0

L7 ANSWER 10 OF 23 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 2000-292877 [25] WPIDS
DOC. NO. NON-CPI: N2000-219631
DOC. NO. CPI: C2000-088472
TITLE: Immunoassay for **equine** protozoal myeloencephalitis in **horses** uses specific **antibodies** to proteins derived from **Sarcocystis neurona**.
DERWENT CLASS: B04 C06 D16 S03
INVENTOR(S): MANSFIELD, L S; MURPHY, A J; ROSSANO, M G
PATENT ASSIGNEE(S): (UNMS) UNIV MICHIGAN STATE
COUNTRY COUNT: 83
PATENT INFORMATION:

| PATENT NO | KIND | DATE | WEEK | LA | PG |
|---|---|---|---|---|---|
| WO 2000017640 | A1 | 20000330 | (200025)* | EN | 26 |

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ UG ZW
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI

09/670355 ; 670096 ; 669843 ; 669833 ; 670244

GB GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS
LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK
SL TJ TM TR TT UA UG UZ VN YU ZW
AU 9954707 A 20000410 (200035)
US 6153394 A 20001128 (200063)

APPLICATION DETAILS:

| PATENT NO | KIND | APPLICATION | DATE |
|---|---|---|---|
| WO 2000017640 | A1 | WO 1999-US17961 | 19990809 |
| AU 9954707 | A | AU 1999-54707 | 19990809 |
| US 6153394 | A | US 1998-156954 | 19980918 |

FILING DETAILS:

| PATENT NO | KIND | | PATENT NO |
|---|---|---|---|
| AU 9954707 | A | Based on | WO 200017640 |

PRIORITY APPLN. INFO: US 1998-156954 19980918
AN 2000-292877 [25] WPIDS
AB WO 200017640 A UPAB: 20000524
NOVELTY - An improved immunoassay for detecting **Sarcocystis neurona** infection in **equines**, comprises reacting the **Sarcocystis neurona** protein with a non-labeled **antibody** to proteins of other Sarcocystis species, before the immunoassay, which inhibits non-specific binding of the labeled **antibody**, during the immunoassay.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a method for the detection of disease caused by Sarcocystis neurons in **equines** which comprises:

(a) isolating fluid from the **equine** which can contain parasite induced **antibodies** to **Sarcocystis neurona** proteins, indicating the presence of the **Sarcocystis neurona**;

(b) reacting the fluid with at least one identifying **antigen** of the Sarcocystis neurons protein bound on a substrate, where the substrate has been blocked with **antibodies** to Sarcocystis sp. other than Sarcocystis neurons, so that **antibodies** to **Sarcocystis neurona antigen** in the fluid are bound to the identifying **antigen**; and

(c) detecting the **antibodies** bound to the **antigen**;

(2) a kit for the detection of disease caused by **Sarcocystis neurona** comprising in separate containers:

(a) an identifying **antibody** able to specifically bind a **Sarcocystis neurona** protein; and

(b) a non-labeled **antibody** which is specific for a second protein of a Sarcocystis sp. other than **Sarcocystis neurona**; and

(3) a kit for the detection of disease caused by **Sarcocystis neurona** in **equines** comprising:

(a) a substrate with at least one identifying **antigen**

to the **Sarcocystis neurona** bound on a surface of the substrate;

(b) **antibody** to a Sarcocystis sp. other than **Sarcocystis neurona**; and

(c) at least one reagent for the detection of an **antibody** in a fluid of the **equine** which binds to the **antigen** of **Sarcocystis neurona**.

USE - The methods and kits are used to detect **antibodies** to proteins of **Sarcocystis neurona**, in an **equine**, (claimed), which causes myeloencephalitis in the **equine**.

ADVANTAGE - The method uses a non-labeled **antibody** to proteins of other Sarcocystis species to inhibit the non-specific binding of the labeled **antibody**, improving the accuracy of the assay.

Dwg.0/2

L7 ANSWER 11 OF 23 MEDLINE
ACCESSION NUMBER: 2001077781 MEDLINE
DOCUMENT NUMBER: 21011431 PubMed ID: 11128499
TITLE: Immunohistochemical confirmation of **Sarcocystis neurona** infections in raccoons, mink, cat, skunk, and pony.
AUTHOR: Dubey J P; Hamir A N
CORPORATE SOURCE: Parasite Biology and Epidemiology Laboratory, Livestock and Poultry Sciences Institute, ARS, USDA, Beltsville, Maryland 20705, USA.
SOURCE: JOURNAL OF PARASITOLOGY, (2000 Oct) 86 (5) 1150-2. Journal code: JL3. ISSN: 0022-3395.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200101
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20010111

AB In the central nervous system of 2 raccoons, 1 cat, 1 pony, 2 mink, and 1 skunk, protozoa previously thought to be Sarcocystis-like reacted positively to **Sarcocystis neurona** -specific **antibodies** in an immunohistochemical test. In addition, **S. neurona** was identified in the brain of another skunk. These observations indicate that **S. neurona** is not confined to opossums and **horses**.

L7 ANSWER 12 OF 23 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1999-571872 [48] WPIDS
DOC. NO. NON-CPI: N1999-421433
DOC. NO. CPI: C1999-166894
TITLE: Biologically pure culture of **equine** Neospora, used as source of vaccines and diagnostic reagents.
DERWENT CLASS: B04 C06 C07 D16 S03
INVENTOR(S): BARR, B C; CONRAD, P A; MARSH, A E
PATENT ASSIGNEE(S): (REGC) UNIV CALIFORNIA
COUNTRY COUNT: 23
PATENT INFORMATION:

09/670355 ; 670096 ; 669843 ; 669833 ; 670244

| PATENT NO | KIND | DATE | WEEK | LA | PG |
|---|---|---|---|---|---|
| WO 9947927 | A1 | 19990923 | (199948)* | EN | 47 |
| RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE | | | | | |
| W: AU CA JP | | | | | |
| AU 9931874 | A | 19991011 | (200008) | | |
| US 6071737 | A | 20000606 | (200033) | | |
| EP 1064550 | A1 | 20010103 | (200102) | EN | |
| R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE | | | | | |

APPLICATION DETAILS:

| PATENT NO | KIND | APPLICATION | DATE |
|---|---|---|---|
| WO 9947927 | A1 | WO 1999-US5754 | 19990316 |
| AU 9931874 | A | AU 1999-31874 | 19990316 |
| US 6071737 | A | US 1998-42600 | 19980316 |
| EP 1064550 | A1 | EP 1999-913906 | 19990316 |
| | | WO 1999-US5754 | 19990316 |

FILING DETAILS:

| PATENT NO | KIND | | PATENT NO |
|---|---|---|---|
| AU 9931874 | A | Based on | WO 9947927 |
| EP 1064550 | A1 | Based on | WO 9947927 |

PRIORITY APPLN. INFO: US 1998-42600 19980316

AN 1999-571872 [48] WPIDS

AB WO 9947927 A UPAB: 19991122

NOVELTY - Biologically pure culture of **equine** Neospora, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(a) detecting **antibodies** (Ab) specifically reactive with **equine** Neospora **antigens** (Ag) by forming an Ab-Ag complex;

(b) detecting Neospora by forming a complex with an **antibody** (Ab1) specifically reactive with Neospora **antigen**;

(c) detecting Neospora-specific nucleic acid (I) by hybridization with a specific oligonucleotide probe; and

(d) pharmaceutical composition containing **equine** Neospora immunogen and a carrier.

ACTIVITY - Antiprotozoal.

MECHANISM OF ACTION - Induction of a specific immune response.

USE - Immunogens (optionally expressed from gene therapy vectors) from **equine** Neospora are used in vaccines for treatment or prevention of Neospora infection in **horses** and other animals. Neospora is a causative agent of **equine** protozoal myeloencephalitis (EPM). Detection of Neospora-specific **antigens, antibodies** or nucleic acid (by usual immunoassay or hybridization tests) is used to diagnose infection. **Antibodies** (Ab) specific for **equine** Neospora are used for diagnosis; to select candidate immunogens for vaccine development; to isolate proteins; to screen DNA libraries and as therapeutic/prophylactic agents.

ADVANTAGE - Reagents specific for **equine** Neospora

allow differentiation between **equine** protozoal myeloencephalitis caused by Neospora and **Sarcocystis neurona**. These pathogens require different treatments and treatment of Neospora is only effective if applied before the parasite has formed cysts. The vaccines also prevent shedding of oocysts by animals known to be infected.
Dwg.0/2

L7 ANSWER 13 OF 23 CABA COPYRIGHT 2001 CABI
ACCESSION NUMBER: 2000:26271 CABA
DOCUMENT NUMBER: 20000804749
TITLE: Prevalence of **antibodies** to Neospora caninum in dogs [sic]
AUTHOR: Cheadle, M. A.; Lindsay, D. S.; Rowe, S.; Dykstra, C. C.; Williams, M. A.; Spencer, J. A.; Toivio-Kinnucan, M. A.; Lenz, S. D.; Newton, J. C.; Rolsma, M. D.; Blagburn, B. L.
CORPORATE SOURCE: Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, AL 36849, USA.
SOURCE: International Journal for Parasitology, (1999) Vol. 29, No. 10, pp. 1537-1543. 25 ref.
Meeting Info.: Neospora caninum and neosporosis.
ISSN: 0020-7519
DOCUMENT TYPE: Journal
LANGUAGE: English
AB An IFAT was used to determine the prevalence of Neospora-specific IgG **antibodies** in serum from asymptomatic **horses** (n=536) from Alabama, USA, which had been routinely submitted for **equine** infectious anaemia virus testing. A 13-year-old **horse** with CNS disease which was seropositive for Neospora was necropsied for the isolation and in vitro cultivation of protozoa. The survey indicated that IgG **antibodies** to Neospora were present in 62 (11.5%) of the 536 serum samples. Endpoint titres for the positive samples were 1:50 (35/6.5%), 1:100 (19/3.5%), 1:200 (7/1.3%) and 1:1600 (1/0.2%). Tachyzoites were first seen in cultured bovine turbinate cells 32 days after inoculation with spinal cord homogenates from the **horse** with CNS disease. The tachyzoites reacted with known N. caninum-positive serum from **horses**, cows, dogs and mice, but did not react with murine anti-Toxoplasma gondii or **equine** anti-**Sarcocystis neurona** serum. Ultrastructural features of the tachyzoites and a comparison of their immunodominant proteins showed that they were identical to those of N. hughesi. The isolate recovered from the **horse** in (designated NA1) is considered to be an isolate of N. hughesi, although additional molecular confirmation is required. The results support the recognition of N. hughesi as a valid species and show that Neospora infections in **horses** may occur in widely separated geographic regions of the USA.

L7 ANSWER 14 OF 23 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 1999441533 MEDLINE
DOCUMENT NUMBER: 99441533 PubMed ID: 10511862
TITLE: Serologic prevalence of **Sarcocystis neurona**, Toxoplasma gondii, and Neospora caninum in **horses** in Brazil.

09/670355 ; 670096 ; 669843 ; 669833 ; 670244

AUTHOR: Dubey J P; Kerber C E; Granstrom D E
CORPORATE SOURCE: Parasite Biology and Epidemiology Laboratory, United States Department of Agriculture, Beltsville Agricultural Research Center, MD 20705-2350, USA.
SOURCE: JOURNAL OF THE AMERICAN VETERINARY MEDICAL ASSOCIATION, (1999 Oct 1) 215 (7) 970-2.
Journal code: HAV; 7503067. ISSN: 0003-1488.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199911
ENTRY DATE: Entered STN: 20000113
Last Updated on STN: 20000113
Entered Medline: 19991130

AB OBJECTIVE: To determine serologic prevalence of **Sarcocystis neurona**, Toxoplasma gondii, and Neospora caninum in **horses** in Brazil. DESIGN: Prevalence survey. ANIMALS: 101 Thoroughbreds in Brazil. PROCEDURE: Blood samples were obtained from **horses** and tested for serum **antibodies** against **S neurona** by use of an immunoblot procedure with culture-derived **S neurona** merozoites as **antigen**, and for serum **antibodies** against T gondii and N caninum by use of a modified agglutination test with formalin-preserved tachyzoites and mercaptoethanol. RESULTS: **Antibodies** against **S neurona** and T gondii were detected in 36 and 16 of 101 **horses**, respectively. Cross-reactivity between **antibodies** against T gondii and **S neurona** was not detected. **Antibodies** against N caninum were not detected in any samples. CONCLUSIONS AND CLINICAL RELEVANCE: The high prevalence of **antibodies** against **S neurona** detected in clinically normal **horses** emphasizes the importance of examining CSF for **antibodies** when establishing a diagnosis of **equine** protozoal myeloencephalitis.

L7 ANSWER 15 OF 23 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 1999417328 MEDLINE
DOCUMENT NUMBER: 99417328 PubMed ID: 10489203
TITLE: Prevalence of **antibodies** to **Sarcocystis neurona**, Toxoplasma gondii and Neospora caninum in **horses** from Argentina.
AUTHOR: Dubey J P; Venturini M C; Venturini L; McKinney J; Pecoraro M
CORPORATE SOURCE: Parasite Biology and Epidemiology Laboratory, United States Department of Agriculture, Agricultural Research Service, Livestock and Poultry Sciences Institute, Beltsville, MD 20705-2350, USA..
jdubey@lpsi.barc.usda.gov
SOURCE: VETERINARY PARASITOLOGY, (1999 Sep 15) 86 (1) 59-62.
Journal code: XBU; 7602745. ISSN: 0304-4017.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199910
ENTRY DATE: Entered STN: 19991101

Last Updated on STN: 19991101
Entered Medline: 19991015

AB Sera from 76 **horses** from Argentina were examined for **antibodies** to **Sarcocystis neurona**, Toxoplasma gondii and Neospora caninum. **Antibodies** to **S. neurona** were found in 27 (35.5%) of 76 **horses** using immunoblots with culture derived merozoites as **antigen. Antibodies** to T. gondii were found in 10 (13.1%) of 76 **horses** by using the modified agglutination test with formalin-fixed tachyzoites and mercaptoethanol; titers were 1:25 (two **horses**), 1:50 (six **horses**), 1:100 (two **horses**), and 1:200 (one **horse**). **Antibodies** to N. caninum were not found in any of the 76 **horses** by the use of N. caninum agglutination test. This is the first report of **S. neurona** infection in **horses** in Argentina.

L7 ANSWER 16 OF 23 CABA COPYRIGHT 2001 CABI
ACCESSION NUMBER: 1998:119288 CABA
DOCUMENT NUMBER: 980805369
TITLE: Evidence that surface proteins Sn14 and Sn16 of **Sarcocystis neurona** merozoites are involved in infection and immunity
AUTHOR: Fang TingLiang; Granstrom, D. E.; Xiao MinZhao; Timoney, J. F.; Xiao, M. Z.
CORPORATE SOURCE: Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, Lexington, KY 40546, USA.
SOURCE: Infection and Immunity, (1998) Vol. 66, No. 5, pp. 1834-1838. 39 ref.
ISSN: 0019-9567
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Based on an analysis of 25 000 **equine** serum and cerebrospinal fluid (CSF) samples at the University of Kentucky, USA, since 1991, including samples from **horses** with neurological signs typical of **equine** protozoal myeloencephalitis (EPM) or with histologically or parasitologically confirmed EPM, 4 major immunoblot band patterns were identified. 23 serum and CSF samples representing each of the 4 immunoblot patterns were selected from 220 samples from **horses** with neurological signs resembling EPM and examined for inhibitory effects on the infectivity of **Sarcocystis neurona** by an in vitro neutralization assay. A high correlation between immunoblot band pattern and neutralizing activity was detected. Two proteins, Sn14 and Sn16 (14 and 16 kDa, respectively), appeared to be important for in vitro infection. A combination of the results of surface protein labelling, immunoprecipitation, Western blotting and trypsin digestion indicated that these molecules are surface proteins. Although **S. neurona** is an obligate intracellular parasite, it is potentially a target for specific **antibodies** which may lyse merozoites via complement or inhibit their attachment and penetration to host cells.

L7 ANSWER 17 OF 23 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 1998384204 EMBASE
TITLE: Neospora caninum-associated **equine**

protozoal myeloencephalitis.
AUTHOR: Hamir A.N.; Tornquist S.J.; Gerros T.C.; Topper M.J.; Dubey J.P.
CORPORATE SOURCE: A.N. Hamir, College of Veterinary Medicine, Oregon State University, Corvallis, OR 97331, United States
SOURCE: Veterinary Parasitology, (1998) 79/4 (269-274). Refs: 12
ISSN: 0304-4017 CODEN: VPARDI
PUBLISHER IDENT.: S 0304-4017(98)00178-2
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English
AB **Equine** protozoal myeloencephalitis (EPM) was clinically diagnosed in a 20-year-old **horse** with severe ataxia. The cerebrospinal fluid was positive for **Sarcocystis neurona antibodies** by western blot. The **horse** was administered corticosteroids to facilitate in vitro culture of **S. neurona** from its spinal cord following necropsy. Microscopic lesions of EPM were present in the brain and in the spinal cord, including multifocal inflammatory cellular infiltrates and several large groups of protozoa. Immunohistochemical, and light and electron microscopic examinations revealed that the protozoa were Neospora caninum and not **S . neurona**. The protozoa divided by endodyogeny, tachyzoites had rhoptries, and organisms reacted specifically to N. caninum **antibodies**. Veterinarians should be aware of increasing diagnosis of N. caninum as another etiological agent responsible for the lesions of EPM. Copyright (C) 1998 Elsevier Science B.V.

L7 ANSWER 18 OF 23 MEDLINE
ACCESSION NUMBER: 97100246 MEDLINE
DOCUMENT NUMBER: 97100246 PubMed ID: 8944807
TITLE: Neosporosis as a cause of **equine** protozoal myeloencephalitis.
AUTHOR: Marsh A E; Barr B C; Madigan J; Lakritz J; Nordhausen R; Conrad P A
CORPORATE SOURCE: Department of Pathology, Microbiology, and Immunology, School of Veterinary Medicine, University of California, Davis 95616-8745, USA.
SOURCE: JOURNAL OF THE AMERICAN VETERINARY MEDICAL ASSOCIATION, (1996 Dec 1) 209 (11) 1907-13.
Journal code: HAV; 7503067. ISSN: 0003-1488.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199701
ENTRY DATE: Entered STN: 19970219
Last Updated on STN: 19970219
Entered Medline: 19970130
AB Neosporosis was diagnosed in an 11-year-old Quarter **Horse** gelding with clinical signs and diagnostic test results compatible with **equine** protozoal myeloencephalitis (EPM). Presumptive postmortem diagnosis of EPM attributable to **Sarcocystis neurona** infection is generally made on the basis of

detecting an **antibody** titer to **S neurona** in the CSF or characteristic histologic lesions, even when parasites have not been specifically identified. Neosporosis was confirmed in the **horse** described here by use of immunohistochemical examination, in vitro culturing, and ultrastructural and molecular characterization of parasites from infected tissues. **Antibody** testing of serum and CSF samples indicated that Neospora-specific anti-bodies can react with **S neurona** proteins on western blot analysis. The confirmation that neosporosis in **horses** can mimic EPM emphasizes the need to broaden the etiologic definition of EPM beyond infections exclusively attributable to **S neurona**.

L7 ANSWER 19 OF 23 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 95:229784 SCISEARCH
THE GENUINE ARTICLE: QN236
TITLE: DIAGNOSIS OF **EQUINE** PROTOZOAL MYELOENCEPHALITIS AND CERVICAL STENOTIC MYELOPATHY
AUTHOR: MOORE B R (Reprint); GRANSTROM D E; REED S M
CORPORATE SOURCE: KANSAS STATE UNIV AGR & APPL SCI, COLL VET MED, DEPT CLIN SCI, MANHATTAN, KS, 66506 (Reprint)
COUNTRY OF AUTHOR: USA
SOURCE: COMPENDIUM ON CONTINUING EDUCATION FOR THE PRACTICING VETERINARIAN, (MAR 1995) Vol. 17, No. 3, pp. 419.
ISSN: 0193-1903.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: AGRI
LANGUAGE: ENGLISH
REFERENCE COUNT: No References Keyed
*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB Advances in cerebrospinal fluid analysis and cervical radiography may improve the ability of the clinician to diagnose **equine** protozoal myeloencephalitis and cervical stenotic myelopathy. Immunoblot analysis is an immunoassay that identifies **antibody** produced in response to **antigens** unique to **Sarcocystis neurona**-the causative agent of **equine** protozoal myeloencephalitis. Positive immunoblot analysis of cerebrospinal fluid indicates parasitic penetration of the blood-brain barrier and intrathecal production of **antibody** to **S. neurona**. Positive immunoblot analysis of serum may be observed in nonataxic **horses** and is not diagnostic for **equine** protozoal myeloencephalitis. To determine the likelihood of cervical stenotic myelopathy, the diameter of the vertebral canal can be accurately assessed from standing cervical radiographs of the **horse** by calculating a proportion of the minimum sagittal diameter of the vertebral canal to the width of the vertebral body (sagittal ratio technique). The accuracy of the sagittal ratio technique for identification of **horses** affected with cervical stenotic myelopathy, without consideration of other bony malformations of the cervical vertebrae, suggests that generalized stenosis of the vertebral canal may be the most important factor in the development of cervical stenotic myelopathy.

L7 ANSWER 20 OF 23 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 93222344 MEDLINE
DOCUMENT NUMBER: 93222344 PubMed ID: 8466988

09/670355 ; 670096 ; 669843 ; 669833 ; 670244

TITLE: **Equine** protozoal myeloencephalitis: **antigen** analysis of cultured **Sarcocystis neurona** merozoites.
AUTHOR: Granstrom D E; Dubey J P; Davis S W; Fayer R; Fox J C; Poonacha K B; Giles R C; Comer P F
CORPORATE SOURCE: Department of Veterinary Science, University of Kentucky, Lexington 40546-0099.
SOURCE: JOURNAL OF VETERINARY DIAGNOSTIC INVESTIGATION, (1993 Jan) 5 (1) 88-90.
Journal code: A2D; 9011490. ISSN: 1040-6387.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199305
ENTRY DATE: Entered STN: 19930521
Last Updated on STN: 19930521
Entered Medline: 19930510

AB **Antigens** of cultured **Sarcocystis neurona** merozoites were examined using immunoblot analysis. Blotted proteins were probed with S. cruzi, S. muris, and **S. neurona** antisera produced in rabbits, S. fayeri (pre- and post-infection) and **S. neurona** (pre- and post-inoculation) sera produced in **horses**, immune sera from 7 histologically confirmed cases of **equine** protozoal myeloencephalitis (EPM), and pre-suckle serum from a newborn foal. Eight proteins, 70, 24, 23.5, 22.5, 13, 11, 10.5, and 10 Kd, were detected only by **S. neurona** antiserum and/or immune serum from EPM-affected **horses**. **Equine** sera were titered by the indirect immunofluorescent **antibody** (IFA) method using air-dried, cultured **S. neurona** merozoites. Anti-Sarcocystis IFA titers were found in **horses** with or without EPM. Serum titers did not correspond to the number of specific bands recognized on immunoblots.

L7 ANSWER 21 OF 23 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 92:618167 SCISEARCH
THE GENUINE ARTICLE: JT862
TITLE: **EQUINE** PROTOZOAL MYELOENCEPHALITIS
AUTHOR: MACKAY R J (Reprint); DAVIS S W; DUBEY J P
CORPORATE SOURCE: UNIV FLORIDA, COLL VET MED, DEPT LARGE ANIM CLIN SCI, GAINESVILLE, FL, 32611 (Reprint)
COUNTRY OF AUTHOR: USA
SOURCE: COMPENDIUM ON CONTINUING EDUCATION FOR THE PRACTICING VETERINARIAN, (OCT 1992) Vol. 14, No. 10, pp. 1359-1367.
ISSN: 0193-1903.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: AGRI
LANGUAGE: ENGLISH
REFERENCE COUNT: No References Keyed
*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB **Equine** protozoal myeloencephalitis is a common focal or multifocal central nervous system disease of **horses** and ponies. The condition was first recognized in the 1960s and has subsequently been reported with increasing frequency. The disease apparently is restricted to North and South America and is more common in the eastern part than the western part of North America.

Searcher : Shears 308-4994

The causative agent, **Sarcocystis neurona**, has recently been identified and is adapted to continuous culture in a bovine monocyte cell line. In the central nervous system of affected **horses**, the organism is found in neural cells and leukocytes in gray and white matter. A carnivorous definitive host for the organism is suspected. The clinical signs of **equine** protozoal myeloencephalitis are extremely variable but are typically referable to asymmetric, multifocal central nervous system disease. Spinal cord lesions caused by **equine** protozoal myeloencephalitis are more common than brain disease, and brain stem signs (e.g., facial paralysis and vestibular signs) occur more frequently than cerebral signs. Although no definitive antemortem diagnostic test is available, the presence of **antibodies** that are cross-reactive with S. cruzi **antigens** is interpreted as supportive of the diagnosis. If untreated, the disease is usually progressive and fatal after a course of days to years. With use of the antiprotozoal agents trimethoprim-sulfadiazine and pyrimethamine, at least 50% of affected **horses** exhibit some improvement; complete recovery is uncommon.

L7 ANSWER 22 OF 23 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 92355818 MEDLINE
DOCUMENT NUMBER: 92355818 PubMed ID: 1644935
TITLE: A five year (1985-1989) retrospective study of **equine** neurological diseases with special reference to rabies.
AUTHOR: Hamir A N; Moser G; Rupprecht C E
CORPORATE SOURCE: Laboratory of Large Animal Pathology, University of Pennsylvania, New Bolton Center, Kennett Square 19348.
CONTRACT NUMBER: AI-09206-16 (NIAID)
SOURCE: JOURNAL OF COMPARATIVE PATHOLOGY, (1992 May) 106 (4) 411-21.
Journal code: HVB; 0102444. ISSN: 0021-9975.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199209
ENTRY DATE: Entered STN: 19920925
Last Updated on STN: 19920925
Entered Medline: 19920910

AB A retrospective study of **horses** necropsied between 1985 and 1989 at a diagnostic laboratory of a veterinary school in North America is documented. In this investigation over 20 per cent of the **horses** had clinical neurological signs. **Equine** protozoal myeloencephalitis (caused by **Sarcocystis neurona**) and cervical stenotic myelopathy (wobbler syndrome) were the most common of these disorders. The veterinary school is located in the midst of a raccoon rabies enzootic area. However, only four cases of **equine** rabies were diagnosed during the 5-year study. The gross microscopical and immunohistochemical findings from these rabies-positive **horses** are documented. Immunoperoxidase tests for detection of rabies **antigen** in another 35 **horses** with non-specific encephalitis/encephalopathy did not reveal any positive cases. Based on this investigation, it appears that immunoperoxidase is a valid

method for diagnosis of rabies when fresh tissues are not available for the fluorescent **antibody** test. It is also concluded that no cases of **equine** rabies were overlooked by the diagnostic laboratory during the period under investigation.

L7 ANSWER 23 OF 23 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1990-179805 [24] WPIDS
DOC. NO. NON-CPI: N1990-139724
DOC. NO. CPI: C1990-078037
TITLE: Monoclonal anti-idiotype **antibodies** - for diagnosis of various infections with no false positive results.
DERWENT CLASS: B04 D16 S03
INVENTOR(S): MOENNIG, V
PATENT ASSIGNEE(S): (MOEN-I) MOENNIG V
COUNTRY COUNT: 1
PATENT INFORMATION:

| PATENT NO | KIND | DATE | WEEK | LA | PG |
|---|---|---|---|---|---|
| DE 3840968 | A | 19900607 | (199024)* | | |
| DE 3840968 | C | 19901004 | (199040) | | |

PRIORITY APPLN. INFO: DE 1988-3840968 19881205
AN 1990-179805 [24] WPIDS
AB DE 3840968 A UPAB: 19930928
A monoclonal anti-idiotypical **antibody** (I) which imitates an epitope of a cause of infection, has the following characteristics:(a) it is generally preserved, (b) it only occurs in serotypes of this cause of infection, (c) it has the capability of inducing the formation of **antibodies** in the natural host, (d) it is part of an immunodominant **antigen**.

A kit for diagnostic purposes, consists of (A) at least one (I) bonded onto a carrier material, which is pref. of plastics, mitrocellulose or dextran spheres, (B) labelled mono- or poly-clonal **antibodies**, effective against immunoglobulins of animal species from which the serum to be inDE3840968A - Cvestigated originates (C) fluorogenic or chromogenic substrate; and (D) a stop soln.

USE/ADVANTAGE - (I) can be used in the diagnosis of various infections, including viral infections (such as European hog cholrea, bovine herpes virus 1, rubella, feline leukaemia, **equine** infectious anaemia, blue tongue, **equine** arthritis), bacterial infections, (such as brucellosis in cattle, shep and pigs, salmonellosis, pasteurellosis) and parasitic infections (such as toxoplasmosis, trichinosis in pigs and **sarcosporidosis**).

(I) enables diagnosis of these and other infections to be carried out, without giving false positive results, as there are no cross-reactions with other causes of infection.

0/2

ABEQ DE 3840968 C UPAB: 19930928
Monoclonal anti-idiotypic **antibody** imitates an epitope of an infectious agent, is genetically conserved, occurs only with serotypes of the infectious agent, induces the formation of **antibodies** in its host environment, and is part of an

immunodominant **antigen**.
USE - These **antibodies** are diagnostic **antigens** for clinical analysis.

FILE 'CAPLUS' ENTERED AT 11:31:15 ON 14 NOV 2001
L8 5 SEA ABB=ON PLU=ON L3 AND (KILOD? OR KILO(W)(DA OR DALTON) OR KD OR KDA OR DALTON)
L9 2 SEA ABB=ON PLU=ON L8 NOT L4

L9 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2001:129328 CAPLUS
DOCUMENT NUMBER: 135:2765
TITLE: Comparison of **Sarcocystis neurona** isolates derived from **horse** neural tissue
AUTHOR(S): Mansfield, L. S.; Schott, H. C.; Murphy, A. J.; Rossano, M. G.; Tanhauser, S. M.; Patterson, J. S.; Nelson, K.; Ewart, S. L.; Marteniuk, J. V.; Bowman, D. D.; Kaneene, J. B.
CORPORATE SOURCE: College of Veterinary Medicine, Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, MI, 48824, USA
SOURCE: Vet. Parasitol. (2001), 95(2-4), 167-178
CODEN: VPARDI; ISSN: 0304-4017
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Sarcocystis neurona** is a protozoan parasite that can cause neurol. deficits in infected **horses**. The route of transmission is by fecal-oral transfer of sporocysts from opossums. However, the species identity and the lifecycle are not completely known. In this study, Sarcocystis merozoites from eight isolates obtained from Michigan **horses** were compared to **S. neurona** from a California **horse** (UCD1), Sarcocystis from a grackle (Cornell), and five Sarcocystis isolates from feral opossums from Michigan.Comparisons were made using several techniques. SDS-PAGE anal. with silver staining showed that Sarcocystis spp. from the eight **horses** appeared the same, but different from the grackle isolate. One Michigan **horse** isolate (MIH6) had two bands at 72 and 25 **kDa** that were more prominent than the UCD1 isolate and other Michigan **horse** isolates. Western blot anal. showed that merozoites of eight of eight **equine**-derived isolates, and the UCD1 **S. neurona** isolate had similar bands when developed with serum or CSF of an infected **horse**. Major bands were seen at 60, 44, 30, and 16 **kDa**. In the grackle (Cornell) isolate, bands were seen at 60, 44, 29, and 16 **kDa**. DNA from merozoites of each of the eight **equine**-derived isolates and the grackle-derived isolate produced a 334 bp PCR product (Tanhauser et al., 1999). Restriction fragment length polymorphism (RFLP) anal. of these **horse** isolates showed banding patterns characteristic for **S. neurona**. The grackle (Cornell) isolate had an RFLP banding pattern characteristic of other S. falcatula species. Finally, electron microscopy examg. multiple merozoites of each of these eight **horse** isolates showed similar morphol., which differed from the grackle (Cornell) isolate. We conclude that the eight Michigan **horse** isolates are **S.**

**neurona** species and the grackle isolate is an S. falcatula species.

REFERENCE COUNT: 18

REFERENCE(S):
(2) Bradford, M; Anal Biochem 1976, V72, P248 CAPLUS
(3) Dame, J; J Parasitol 1995, V81, P930 CAPLUS
(4) Dubey, J; J Parasitol 1991, V77, P212 MEDLINE
(8) Fenger, C; J Parasitol 1995, V81, P916 CAPLUS
(18) Tanhauser, S; J Parasitol 1999, V85, P221 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1997:468598 CAPLUS
DOCUMENT NUMBER: 127:217372
TITLE: Micropreparative high resolution purification of proteins by a combination of sodium dodecyl sulfate-polyacrylamide gel electrophoresis, isoelectric focusing, and membrane blotting
AUTHOR(S): Liang, Fang Ting; Granstrom, David E.; Timoney, John F.; Shi, Yu Fang
CORPORATE SOURCE: Gluck Equine Research Center, Dep. of Veterinary Science, University of Kentucky, Lexington, KY, 40546, USA
SOURCE: Anal. Biochem. (1997), 250(1), 61-65
CODEN: ANBCA2; ISSN: 0003-2697
PUBLISHER: Academic
DOCUMENT TYPE: Journal
LANGUAGE: English
AB We report a simple, economical, and efficient protocol for protein purifn. from cells. First, proteins of cell lysates were sepd. by std. SDS-PAGE and electroblotted to protein-blotting membrane. The blots were stained with Coomassie blue or developed by immunoblotting to visualize specific proteins. The bands corresponding to those visible by immunoblotting were excised from the dye-stained blots and subjected to isoelec. focusing. The focused gel was stained with Coomassie blue. Finally, the stained bands were excised and subjected to another SDS-PAGE sepn. and electrotransferred back to protein-blotting membrane. At this stage, the purified proteins were suitable for microsequencing. We have tested the feasibility of this novel technique by purifying proteins with mol. wts. ranging from 19 to 100 **kDa** from a lysate of **Sarcocystis neurona**, the etiol. agent of **equine** protozoal myeloencephalitis. The purity of proteins was demonstrated by reverse-phase high-performance liq. chromatog. Partial sequences of these purified proteins were obtained by N-terminal or digestive sequencing.

(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, JAPIO, CABA, AGRICOLA, VETU, VETB' ENTERED AT 11:33:53 ON 14 NOV 2001)
L10 26 S L8
L11 19 S L10 NOT L6
L12 5 DUP REM L11 (14 DUPLICATES REMOVED)

L12 ANSWER 1 OF 5 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 1

09/670355 ; 670096 ; 669843 ; 669833 ; 670244

ACCESSION NUMBER: 2001:169887 BIOSIS
DOCUMENT NUMBER: PREV200100169887
TITLE: Immunoconversion against **Sarcocystis neurona** in normal and dexamethasone-treated **horses** challenged with **S. neurona** sporocysts.
AUTHOR(S): Cutler, Tim J.; MacKay, Robert J. (1); Ginn, Pamela E.; Gillis, Karen; Tanhauser, Susan M.; LeRay, Erin V.; Dame, John B.; Greiner, Ellis C.
CORPORATE SOURCE: (1) Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL, 32610: mackayr@mail.vetmed.ufl.edu USA
SOURCE: Veterinary Parasitology, (26 February, 2001) Vol. 95, No. 2-4, pp. 197-210. print.
ISSN: 0304-4017.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English

AB **Equine** protozoal myeloencephalitis is a common neurologic disease of **horses** in the Americas usually caused by **Sarcocystis neurona**. To date, the disease has not been induced in **horses** using characterized sporocysts from Didelphis virginiana, the definitive host. **S. neurona** sporocysts from 15 naturally infected opossums were fed to **horses** seronegative for antibodies against **S. neurona**. Eight **horses** were given 5 X 105 sporocysts daily for 7 days. **Horses** were examined for abnormal clinical signs, and blood and cerebrospinal fluid were harvested at intervals for 90 days after the first day of challenge and analyzed both qualitatively (western blot) and quantitatively (anti-17 **kDa**) for anti-**S. neurona** IgG. Four of the challenged **horses** were given dexamethasone (0.1 mg/kg orally once daily) for the duration of the experiment. All challenged **horses** immunoconverted against **S. neurona** in blood within 32 days of challenge and in CSF within 61 days. There was a trend ($P = 0.057$) for **horses** given dexamethasone to immunoconvert earlier than **horses** that were not immunosuppressed. Anti-17 **kDa** was detected in the CSF of all challenged **horses** by day 61. This response was statistically greater at day 32 in **horses** given dexamethasone. Control **horses** remained seronegative throughout the period in which all challenged **horses** converted. One control **horse** immunoconverted in blood at day 75 and in CSF at day 89. Signs of neurologic disease were mild to equivocal in challenged **horses**. **Horses** given dexamethasone had more severe signs of limb weakness than did **horses** not given dexamethasone; however, we could not determine whether these signs were due to spinal cord disease or to effects of systemic illness. At necropsy, mild-moderate multifocal gliosis and neurophagia were found histologically in the spinal cords of 7/8 challenged **horses**. No organisms were seen either in routinely processed sections or by immunohistochemistry. Although neurologic disease comparable to naturally occurring **equine** protozoal myeloencephalitis (EPM) was not produced, we had clear evidence of an immune response to challenge both systemically and in the CNS. Broad immunosuppression with dexamethasone did not increase the severity of histologic changes in

the CNS of challenged **horses**. Future work must focus on defining the factors that govern progression of inapparent **S**. **neurona** infection to EPM.

L12 ANSWER 2 OF 5 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 2
ACCESSION NUMBER: 2001:169884 BIOSIS
DOCUMENT NUMBER: PREV200100169884
TITLE: Comparison of **Sarcocystis neurona** isolates derived from **horse** neural tissue.
AUTHOR(S): Mansfield, L. S. (1); Schott, H. C., II; Murphy, A. J.; Rossano, M. G.; Tanhauser, S. M.; Patterson, J. S.; Nelson, K.; Ewart, S. L.; Marteniuk, J. V.; Bowman, D. D.; Kaneene, J. B.
CORPORATE SOURCE: (1) Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, MI, 48824: mansfie4@cvm.msu.edu USA
SOURCE: Veterinary Parasitology, (26 February, 2001) Vol. 95, No. 2-4, pp. 167-178. print.
ISSN: 0304-4017.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB **Sarcocystis neurona** is a protozoan parasite that can cause neurological deficits in infected **horses**. The route of transmission is by fecal-oral transfer of sporocysts from opossums. However, the species identity and the lifecycle are not completely known. In this study, Sarcocystis merozoites from eight isolates obtained from Michigan **horses** were compared to **S**. **neurona** from a California **horse** (UCD1), Sarcocystis from a grackle (Cornell), and five Sarcocystis isolates from feral opossums from Michigan. Comparisons were made using several techniques. SDS-PAGE analysis with silver staining showed that Sarcocystis spp. from the eight **horses** appeared the same, but different from the grackle isolate. One Michigan **horse** isolate (MIH6) had two bands at 72 and 25 **kDa** that were more prominent than the UCD1 isolate and other Michigan **horse** isolates. Western blot analysis showed that merozoites of eight of eight **equine**-derived isolates, and the UCD1 **S**. **neurona** isolate had similar bands when developed with serum or CSF of an infected **horse**. Major bands were seen at 60, 44, 30, and 16 **kDa**. In the grackle (Cornell) isolate, bands were seen at 60, 44, 29, and 16 **kDa**. DNA from merozoites of each of the eight **equine**-derived isolates and the grackle-derived isolate produced a 334 bp PCR product (Tanhauser et al., 1999). Restriction fragment length polymorphism (RFLP) analysis of these **horse** isolates showed banding patterns characteristic for **S**. **neurona**. The grackle (Cornell) isolate had an RFLP banding pattern characteristic of other S. falcatula species. Finally, electron microscopy examining multiple merozoites of each of these eight **horse** isolates showed similar morphology, which differed from the grackle (Cornell) isolate. We conclude that the eight Michigan **horse** isolates are **S**. **neurona** species and the grackle isolate is an S. falcatula species.

L12 ANSWER 3 OF 5 MEDLINE DUPLICATE 3

ACCESSION NUMBER: 2000152631 MEDLINE
DOCUMENT NUMBER: 20152631 PubMed ID: 10690772
TITLE: Improvement of western blot test specificity for detecting **equine** serum antibodies to **Sarcocystis neurona**.
AUTHOR: Rossano M G; Mansfield L S; Kaneene J B; Murphy A J; Brown C M; Schott H C 2nd; Fox J C
CORPORATE SOURCE: Animal Health Diagnostic Laboratory, The Population Medicine Center, Michigan State University, East Lansing 48824, USA.
SOURCE: JOURNAL OF VETERINARY DIAGNOSTIC INVESTIGATION, (2000 Jan) 12 (1) 28-32.
Journal code: A2D; 9011490. ISSN: 1040-6387.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200003
ENTRY DATE: Entered STN: 20000330
Last Updated on STN: 20000330
Entered Medline: 20000321

AB **Equine** protozoal myeloencephalitis (EPM) is a neurological disease of **horses** and ponies caused by the apicomplexan protozoan parasite **Sarcocystis neurona**. The purposes of this study were to develop the most stringent criteria possible for a positive test result, to estimate the sensitivity and specificity of the EPM Western blot antibody test, and to assess the ability of bovine antibodies to Sarcocystis cruzi to act as a blocking agent to minimize false-positive results in the western blot test for **S. neurona**. **Sarcocystis neurona** merozoites harvested from **equine** dermal cell culture were heat denatured, and the proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in a 12-20% linear gradient gel. Separated proteins were electrophoretically transferred to polyvinylidene fluoride membranes and blocked in 1% bovine serum albumin and 0.5% Tween-Tris-buffered saline. Serum samples from 6 **horses** with **S. neurona** infections (confirmed by culture from neural tissue) and 57 **horses** without infections (**horses** from the Eastern Hemisphere, where **S. neurona** does not exist) were tested by Western blot. **Horses** from both groups had reactivity to the 62-, 30-, 16-, 13-, 11-, 10.5-, and 10-**kD** bands. Testing was repeated with another step. Blots were treated with bovine S. cruzi antibodies prior to loading the **equine** samples. After this modification of the Western blot test, positive infection status was significantly associated with reactivity to the 30- and 16-**kD** bands ($P<0.001$, Fisher's exact test). The S. cruzi antibody-blocked Western blot had a sample sensitivity of 100% and sample specificity of 98%. It is concluded that the specificity of the Western blot test is improved by blocking proteins not specific to **S. neurona** and using reactivity to the 30- and 16-**kD** bands as the criterion for a positive test.

L12 ANSWER 4 OF 5 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 1998234002 MEDLINE
DOCUMENT NUMBER: 98234002 PubMed ID: 9573058
TITLE: Evidence that surface proteins Sn14 and Sn16 of

09/670355 ; 670096 ; 669843 ; 669833 ; 670244

**Sarcocystis neurona** merozoites are involved in infection and immunity.

AUTHOR: Liang F T; Granstrom D E; Zhao X M; Timoney J F

CORPORATE SOURCE: Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, Lexington 40546-0099, USA.

SOURCE: INFECTION AND IMMUNITY, (1998 May) 66 (5) 1834-8. Journal code: GO7; 0246127. ISSN: 0019-9567.

PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199805

ENTRY DATE: Entered STN: 19980520
Last Updated on STN: 19980520
Entered Medline: 19980514

AB **Sarcocystis neurona** is the etiologic agent of **equine** protozoal myeloencephalitis (EPM). Based on an analysis of 25,000 **equine** serum and cerebrospinal fluid (CSF) samples, including samples from **horses** with neurologic signs typical of EPM or with histologically or parasitologically confirmed EPM, four major immunoblot band patterns have been identified. Twenty-three serum and CSF samples representing each of the four immunoblot patterns were selected from 220 samples from **horses** with neurologic signs resembling EPM and examined for inhibitory effects on the infectivity of **S. neurona** by an in vitro neutralization assay. A high correlation between immunoblot band pattern and neutralizing activity was detected. Two proteins, Sn14 and Sn16 (14 and 16 **kDa**, respectively), appeared to be important for in vitro infection. A combination of the results of surface protein labeling, immunoprecipitation, Western blotting, and trypsin digestion suggests that these molecules are surface proteins and may be useful components of a vaccine against **S. neurona** infection. Although **S. neurona** is an obligate intracellular parasite, it is potentially a target for specific antibodies which may lyse merozoites via complement or inhibit their attachment and penetration to host cells.

L12 ANSWER 5 OF 5 MEDLINE DUPLICATE 5

ACCESSION NUMBER: 97378218 MEDLINE

DOCUMENT NUMBER: 97378218 PubMed ID: 9234899

TITLE: Micropreparative high resolution purification of proteins by a combination of sodium dodecyl sulfate-polyacrylamide gel electrophoresis, isoelectric focusing, and membrane blotting.

AUTHOR: Liang F T; Granstrom D E; Timoney J F; Shi Y F

CORPORATE SOURCE: Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, Lexington 40546, USA.

SOURCE: ANALYTICAL BIOCHEMISTRY, (1997 Jul 15) 250 (1) 61-5. Journal code: 4NK; 0370535. ISSN: 0003-2697.

PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199709

ENTRY DATE: Entered STN: 19970916

Searcher : Shears 308-4994

09/670355 ; 670096 ; 669843 ; 669833 ; 670244

Last Updated on STN: 19970916
Entered Medline: 19970904

AB We report a simple, economical, and efficient protocol for protein purification from cells. First, proteins of cell lysates were separated by standard sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and electroblotted to protein-blotting membrane. The blots were stained with Coomassie blue or developed by immunoblotting to visualize specific proteins. The bands corresponding to those visible by immunoblotting were excised from the dye-stained blots and subjected to isoelectric focusing. The focused gel was stained with Coomassie blue. Finally, the stained bands were excised and subjected to another SDS-PAGE separation and electrotransferred back to protein-blotting membrane. At this stage, the purified proteins were suitable for microsequencing. We have tested the feasibility of this novel technique by purifying proteins with molecular weights ranging from 19 to 100 **kDa** from a lysate of **Sarcocystis neurona**, the etiologic agent of **equine** protozoal myeloencephalitis. The purity of proteins was demonstrated by reverse-phase high-performance liquid chromatography. Partial sequences of these purified proteins were obtained by N-terminal or digestive sequencing.

(FILE 'MEDLINE' ENTERED AT 11:37:32 ON 14 NOV 2001)

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|---|---|---|---|---|---|---|
| L13 | 36589 | SEA | FILE=MEDLINE | ABB=ON | PLU=ON | HORSES/CT |
| L14 | 922 | SEA | FILE=MEDLINE | ABB=ON | PLU=ON | SARCOCYSTIS/CT |
| L15 | 85 | SEA | FILE=MEDLINE | ABB=ON | PLU=ON | L13 AND L14 |
| L16 | 57191 | SEA | FILE=MEDLINE | ABB=ON | PLU=ON | ANTIBODIES/CT |
| L17 | 0 | SEA | FILE=MEDLINE | ABB=ON | PLU=ON | L15 AND L16 |

| | | | | | | |
|---|---|---|---|---|---|---|
| L14 | 922 | SEA | FILE=MEDLINE | ABB=ON | PLU=ON | SARCOCYSTIS/CT |
| L18 | 319 | SEA | FILE=MEDLINE | ABB=ON | PLU=ON | EQUIDAE/CT |
| L19 | 5 | SEA | FILE=MEDLINE | ABB=ON | PLU=ON | L18 AND L14 |

| | | | | | | |
|---|---|---|---|---|---|---|
| L13 | 36589 | SEA | FILE=MEDLINE | ABB=ON | PLU=ON | HORSES/CT |
| L14 | 922 | SEA | FILE=MEDLINE | ABB=ON | PLU=ON | SARCOCYSTIS/CT |
| L15 | 85 | SEA | FILE=MEDLINE | ABB=ON | PLU=ON | L13 AND L14 |
| L20 | 47476 | SEA | FILE=MEDLINE | ABB=ON | PLU=ON | ANTIGENS/CT |
| L21 | 0 | SEA | FILE=MEDLINE | ABB=ON | PLU=ON | L15 AND L20 |

L19 ANSWER 1 OF 5 MEDLINE
AN 2001140655 MEDLINE
TI The seroprevalence of antibodies to Sarcocystis neurona in Michigan equids.
AU Rossano M G; Kaneene J B; Marteniuk J V; Banks B D; Schott H C; Mansfield L S
SO PREVENTIVE VETERINARY MEDICINE, (2001 Jan 29) 48 (2) 113-28. Journal code: CWT; 8217463. ISSN: 0167-5877.
AB A cross-sectional study of serum antibodies to Sarcocystis neurona (the etiologic agent of equine protozoal myeloencephalitis, EPM) was performed on Michigan equids. Our objectives were to determine the seroprevalence of antibodies to S. neurona in Michigan equids and to identify specific risk factors for seropositivity. A random, weighted sample of Michigan horse farms (stratified by the state's opossum (Didelphis virginiana) population and the number of equids on each operation) was selected. Ninety-eight equine-operation owners agreed to participate, and blood collection occurred from

late March through October of 1997. Data regarding the 98 farms' feeding and management practices were collected, as well as descriptive data for each of the 1121 individual horses. Serum samples were tested for antibodies to S. neurona using a Western blot test. The true seroprevalence of antibodies specific to S. neurona was estimated to be 60%. Chi-square analysis showed that seroprevalence was lowest in the colder parts of the state that had the fewest opossums (P<0.0001). In two multivariable logistic-regression analyses with random effects grouped by herd, age and exposure to pasture were associated with increased odds of seropositivity, and feeding of sweet feed (grains mixed with molasses) was associated with decreased odds of testing positive. No association was found between farm size, animal gender, hay types, horse-housing types or exposure to natural surface water and seropositivity.

L19 ANSWER 2 OF 5 MEDLINE
AN 2001047783 MEDLINE
TI Detection of Sarcocystis neurona in the brain of a Grant's zebra (Equus burchelli bohmi).
AU Marsh A E; Denver M; Hill F I; McElhaney M R; Trupkiewicz J G; Stewart J; Tell L
SO JOURNAL OF ZOO AND WILDLIFE MEDICINE, (2000 Mar) 31 (1) 82-6. Journal code: CWI. ISSN: 1042-7260.
AB An 8-yr-old intact male Grant's zebra (Equus burchelli bohmi) was referred to the Veterinary Medical Teaching Hospital of the University of California-Davis after being found in the owner's pasture obtunded and in lateral recumbency. The animal was hypothermic, weak, and unwilling to rise. There was no evidence of trauma, and the zebra had seemed normal the preceding evening. There was no extensor rigidity, and cranial nerve reflexes were normal. Flexor and extensor reflexes were weak upon initial examination. A complete blood count and serum biochemistry analysis revealed a mild leukocytosis, hyperfibrinogenemia, hypoglycemia, hyponatremia, hypochloremia, hypocalcemia, and hypoalbuminemia. Urinalysis was normal, and a urine toxicology screen for alkaloids was negative. No toxic substance was found in the hay or pasture grasses although the owner reported the presence of yellow star thistle and mushrooms in the pasture. The cerebrospinal fluid cytologic and biochemical analyses were normal, but antibodies to Sarcocystis neurona were detected. The zebra died despite aggressive supportive therapy over a 4-day period. The necropsy demonstrated severe gastrointestinal nematodiasis that could account for hypoalbuminemia and electrolyte abnormalities. Histopathologic examination of the nervous system revealed focal areas of perivascular cuffing in the brainstem that were comprised mainly of lymphocytes, monocytes, and plasma cells. Immunohistochemical staining identified the presence of S. neurona merozoites associated with the lesions. This zebra probably died from severe endoparasitism that resulted in malabsorption, weakness, and recumbency rather than from encephalitis associated with S. neurona merozoites. Equine protozoal myeloencephalitis has not been reported previously in nondomestic equids.

L19 ANSWER 3 OF 5 MEDLINE
AN 2001023119 MEDLINE
TI Inoculation of Sarcocystis neurona merozoites into the central nervous system of horses.
AU Lindsay D S; Dykstra C C; Williams A; Spencer J A; Lenz S D; Palma

K; Dubey J P; Blagburn B L
SO VETERINARY PARASITOLOGY, (2000 Sep 20) 92 (2) 157-63.
Journal code: XBU. ISSN: 0304-4017.
AB Equine protozoal myeloencephalitis (EPM) is a neurologic syndrome in horses from the Americas and is usually caused by infection with the apicomplexan parasite, Sarcocystis neurona. A horse model of EPM is needed to test the efficacy of chemotherapeutic agents and potential vaccines. Five horses that were negative for antibodies to S. neurona in their serum and cerebrospinal fluid (CSF) were injected in the subarachnoid space with living merozoites of the SN2 isolate of S. neurona. None of the horses developed clinical disease or died over a 132-day observation period. All five horses developed antibodies to S. neurona in their CSF and serum 3-4 weeks after injection. Two of the horses were examined at necropsy and no parasite induced lesions were observed in their tissues and no parasites were recovered from portions of their spinal cords inoculated on to cell cultures. Results of this study demonstrate that merozoites of the SN2 isolate of S. neurona will induce seroconversion but not clinical disease when inoculated directly into the CSF of nonimmune horses.

L19 ANSWER 4 OF 5 MEDLINE
AN 1998430858 MEDLINE
TI Pig, donkey and buffalo meat as a source of some coccidian parasites infecting dogs.
AU Zayed A A; El-Ghaysh A
SO VETERINARY PARASITOLOGY, (1998 Aug 14) 78 (3) 161-8.
Journal code: XBU; 7602745. ISSN: 0304-4017.
AB Experimental infection of dogs with meat samples (oesophagus, heart and diaphragm) from each of 105 pigs, 11 donkeys and 17 Egyptian water buffaloes indicated that they contained the infective stages of some coccidian parasites of dogs. The dogs which were fed pig meat shed in their faeces Isospora ohioensis, I. canis oocysts and Sarcocystis miescheriana sporocysts after prepatent periods of 3-5, 4-7 and 9-10 days, respectively. The dogs which were fed donkey meat excreted only I. ohioensis oocysts and Sarcocystis bertrami sporocysts after prepatent periods of 3 and 11 days, respectively. However, the dogs which were fed buffalo meat shed in their faeces I. ohioensis, I. canis and Hammondia heydorni oocysts with prepatent periods of 1, 1 and 7 days, respectively.

L19 ANSWER 5 OF 5 MEDLINE
AN 97077402 MEDLINE
TI Prevalence of sarcocysts in livestock of northwest Ethiopia.
AU Woldemeskel M; Gebreab F
SO ZENTRALBLATT FUR VETERINARMEDIZIN. REIHE B, (1996 Mar) 43 (1) 55-8.
Journal code: Y72; 0331325. ISSN: 0514-7166.
AB A survey of Sarcocystis was conducted in cattle, sheep, goats, donkeys and chickens. A total of 671 haematoxylin-eosin (H-E) stained muscle tissue samples, including diaphragm, masseter, cardiac and oesophageal musculatures were examined. Additionally, cardiac muscle samples from 40 fetuses were included. An infestation rate of 93% in sheep, 82% in cattle, 81% in goats, 16.6% in donkeys and 6.6% in chickens was noted. The infestation rate of diaphragm, masseter, cardiac and oesophageal musculatures seems to be similar. None of the 40 fetal heart muscle samples from bovine, ovine, caprine and donkey fetuses examined harboured Sarcocystis. An attempt was made to demonstrate the possible occurrence of human

Sarcocystis and a negative result was obtained. The possible impact of Sarcocystis on animal health in Ethiopia is discussed.

(FILE 'CAPLUS, MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, JAPIO, CABA, AGRICOLA, VETU, VETB' ENTERED AT 11:41:15 ON 14 NOV 2001)

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L22         712 S MANSFIELD L?/AU
L23          60 S ROSSANO M?/AU
L24        3767 S MURPHY A?/AU
L25          36 S VRABLE R?/AU
L26           4 S L22 AND L23 AND L24 AND L25
L27          40 S L22 AND (L23 OR L24 OR L25)
L28          19 S L23 AND (L24 OR L25)
L29           4 S L24 AND L25
L30        4512 S L22 OR L23 OR L24 OR L25
L31          28 S (L27 OR L30) AND L3
L32          29 S L26 OR L28 OR L29 OR L31
L33           9 DUP REM L32 (20 DUPLICATES REMOVED)
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- Author(s)

L33 ANSWER 1 OF 9 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 1

ACCESSION NUMBER: 2001:167817 CAPLUS
DOCUMENT NUMBER: 134:221431
TITLE: Vaccine to control **equine** protozoal myeloencephalitis in **horses**
INVENTOR(S): **Mansfield, Linda S.; Rossano, Mary G.; Murphy, Alice J.; Vrable, Ruth A.**
PATENT ASSIGNEE(S): Michigan State University, USA
SOURCE: PCT Int. Appl., 57 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 2001015708 | A1 | 20010308 | WO 2000-US24221 | 20000831 |

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 1999-152193 P 19990902
US 2000-513086 A 20000224

AB The present invention provides vaccines and methods for making the vaccines that actively or passively protect an **equid** or other animal against **Sarcocystis neurona**. In particular, the present invention provides vaccines that provide active immunity which comprise a polypeptide or DNA vaccine that contains or expresses at least one epitope of an antigen that has an amino acid sequence substantially similar to a unique 16 (+/-4) kDa antigen and/or 30 (+/-4) kDa antigen of **Sarcocystis neurona**. The present invention further provides a vaccine

that provides passive immunity to **Sarcocystis neurona** comprising polyclonal or monoclonal antibodies against at least one epitope of an antigen substantially similar to a unique 16 (+/-4) kDa antigen and/or 30 (+/-4) kDa antigen of **Sarcocystis neurona**.

REFERENCE COUNT: 1
REFERENCE(S): (1) Liang; Infection and Immunity 1998, V66(5), P1834 CAPLUS

L33 ANSWER 2 OF 9 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 2
ACCESSION NUMBER: 2001:129328 CAPLUS
DOCUMENT NUMBER: 135:2765
TITLE: Comparison of **Sarcocystis neurona** isolates derived from **horse** neural tissue
AUTHOR(S): **Mansfield, L. S.**; Schott, H. C.; **Murphy, A. J.**; **Rossano, M. G.**; Tanhauser, S. M.; Patterson, J. S.; Nelson, K.; Ewart, S. L.; Marteniuk, J. V.; Bowman, D. D.; Kaneene, J. B.
CORPORATE SOURCE: College of Veterinary Medicine, Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, MI, 48824, USA
SOURCE: Vet. Parasitol. (2001), 95(2-4), 167-178
CODEN: VPARDI; ISSN: 0304-4017
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Sarcocystis neurona** is a protozoan parasite that can cause neurol. deficits in infected **horses**. The route of transmission is by fecal-oral transfer of sporocysts from opossums. However, the species identity and the lifecycle are not completely known. In this study, Sarcocystis merozoites from eight isolates obtained from Michigan **horses** were compared to **S. neurona** from a California **horse** (UCD1), Sarcocystis from a grackle (Cornell), and five Sarcocystis isolates from feral opossums from Michigan.Comparisons were made using several techniques. SDS-PAGE anal. with silver staining showed that Sarcocystis spp. from the eight **horses** appeared the same, but different from the grackle isolate. One Michigan **horse** isolate (MIH6) had two bands at 72 and 25 kDa that were more prominent than the UCD1 isolate and other Michigan **horse** isolates. Western blot anal. showed that merozoites of eight of eight **equine**-derived isolates, and the UCD1 **S. neurona** isolate had similar bands when developed with serum or CSF of an infected **horse**. Major bands were seen at 60, 44, 30, and 16 kDa. In the grackle (Cornell) isolate, bands were seen at 60, 44, 29, and 16 kDa. DNA from merozoites of each of the eight **equine**-derived isolates and the grackle-derived isolate produced a 334 bp PCR product (Tanhauser et al., 1999). Restriction fragment length polymorphism (RFLP) anal. of these **horse** isolates showed banding patterns characteristic for **S. neurona**. The grackle (Cornell) isolate had an RFLP banding pattern characteristic of other S. falcatula species. Finally, electron microscopy examg. multiple merozoites of each of these eight **horse** isolates showed similar morphol., which differed from the grackle (Cornell) isolate. We conclude that the eight Michigan

**horse** isolates are **S. neurona** species and the grackle isolate is an S. falcatula species.

REFERENCE COUNT: 18

REFERENCE(S):
(2) Bradford, M; Anal Biochem 1976, V72, P248 CAPLUS
(3) Dame, J; J Parasitol 1995, V81, P930 CAPLUS
(4) Dubey, J; J Parasitol 1991, V77, P212 MEDLINE
(8) Fenger, C; J Parasitol 1995, V81, P916 CAPLUS
(18) Tanhauser, S; J Parasitol 1999, V85, P221 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L33 ANSWER 3 OF 9 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 2001140655 MEDLINE
DOCUMENT NUMBER: 21068737 PubMed ID: 11154784
TITLE: The seroprevalence of antibodies to **Sarcocystis neurona** in Michigan **equids**.
AUTHOR: **Rossano M G**; Kaneene J B; Marteniuk J V; Banks B D; Schott H C; **Mansfield L S**
CORPORATE SOURCE: The Population Medicine Center, College of Veterinary Medicine, A-109 Veterinary Medical Center, Michigan State University, 48824-1314, East Lansing, MI, USA.
SOURCE: PREVENTIVE VETERINARY MEDICINE, (2001 Jan 29) 48 (2) 113-28.
Journal code: CWT; 8217463. ISSN: 0167-5877.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200103
ENTRY DATE: Entered STN: 20010404
Last Updated on STN: 20010404
Entered Medline: 20010308

AB A cross-sectional study of serum antibodies to **Sarcocystis neurona** (the etiologic agent of **equine** protozoal myeloencephalitis, EPM) was performed on Michigan **equids**. Our objectives were to determine the seroprevalence of antibodies to **S. neurona** in Michigan **equids** and to identify specific risk factors for seropositivity. A random, weighted sample of Michigan **horse** farms (stratified by the state's opossum (Didelphis virginiana) population and the number of **equids** on each operation) was selected. Ninety-eight **equine**-operation owners agreed to participate, and blood collection occurred from late March through October of 1997. Data regarding the 98 farms' feeding and management practices were collected, as well as descriptive data for each of the 1121 individual **horses**. Serum samples were tested for antibodies to **S. neurona** using a Western blot test. The true seroprevalence of antibodies specific to **S. neurona** was estimated to be 60%. Chi-square analysis showed that seroprevalence was lowest in the colder parts of the state that had the fewest opossums (P<0.0001). In two multivariable logistic-regression analyses with random effects grouped by herd, age and exposure to pasture were associated with increased odds of seropositivity, and feeding of sweet feed (grains mixed with

molasses) was associated with decreased odds of testing positive. No association was found between farm size, animal gender, hay types, **horse**-housing types or exposure to natural surface water and seropositivity.

L33 ANSWER 4 OF 9 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 4
ACCESSION NUMBER: 2000:592749 CAPLUS
DOCUMENT NUMBER: 133:191998
TITLE: An antigen test to detect **equine** protozoal myeloencephalitis in **horse** serum and cerebrospinal fluid
INVENTOR(S): **Mansfield, Linda S.; Rossano, Mary G.; Murphy, Alice J.; Vrable, Ruth A.**
PATENT ASSIGNEE(S): Michigan State University, USA
SOURCE: PCT Int. Appl., 64 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 2000049049 | A1 | 20000824 | WO 2000-US4379 | 20000218 |

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 1999-120831 P 19990219
US 1999-152193 P 19990902

AB The present invention provides an immunoassay to detect identifying antigens in **horses** that are infected with **Sarcocystis neurona** . The immunoassay is preferably an antigen-capture-based assay that relies upon polyclonal or monoclonal antibodies against a 16 (<u4) and/or 30 (<u4) kDa antigens specific to **Sarcocystis neurona** to detect the presence of the 16 (<u4) and/or 30 (<u4) kDa antigens in **equine** serum or **equine** cerebrospinal fluid.

REFERENCE COUNT: 3
REFERENCE(S):
(1) Catty; Antibodies Volume II a practical approach 1989, P97
(2) Goding, J; Moloclonal Antibodies:Principles and Practice London 1983, P56
(3) Liang; Infection and Immunity 1998, V66(5), P1834 CAPLUS

L33 ANSWER 5 OF 9 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 5
ACCESSION NUMBER: 2000:210497 CAPLUS
DOCUMENT NUMBER: 132:250014
TITLE: Immunoassay for **equine** protozoal myeloencephalitis in **horses**
INVENTOR(S): **Mansfield, Linda S.; Murphy,**

09/670355 ; 670096 ; 669843 ; 669833 ; 670244

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| | **Alice J.; Rossano, Mary G.** |
| PATENT ASSIGNEE(S): | Michigan State University, USA |
| SOURCE: | PCT Int. Appl., 26 pp. |
| | CODEN: PIXXD2 |
| DOCUMENT TYPE: | Patent |
| LANGUAGE: | English |
| FAMILY ACC. NUM. COUNT: | 1 |
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| WO 2000017640 | A1 | 20000330 | WO 1999-US17961 | 19990809 |
| W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | | |
| RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG | | | | |
| US 6153394 | A | 20001128 | US 1998-156954 | 19980918 |
| AU 9954707 | A1 | 20000410 | AU 1999-54707 | 19990809 |

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| | WO 1999-US17961 | W | 19990809 |

AB An immunoassay for **Sarcocystis neurona** antibodies in **equines** is described. The immunoassay uses blocking of Sarcocystis antigens by antibodies to Sarcocystis sp. other than **Sarcocystis neurona** in connection with the immunoassay.

REFERENCE COUNT: 4

REFERENCE(S):
(1) Boyer; US 5399484 A 1995 CAPLUS
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L33 ANSWER 6 OF 9 BIOSIS COPYRIGHT 2001 BIOSIS

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|---|---|
| ACCESSION NUMBER: | 2001:258224 BIOSIS |
| DOCUMENT NUMBER: | PREV200100258224 |
| TITLE: | Immunoassay for **equine** protozoal myeloencephalitis in **horses**. |
| AUTHOR(S): | **Mansfield, Linda S. (1); Murphy, Alice J.; Rossano, Mary G.** |
| CORPORATE SOURCE: | (1) Bath, MI USA<br>ASSIGNEE: Board of Trustees operating Michigan State University |
| PATENT INFORMATION: | US 6153394 November 28, 2000 |
| SOURCE: | Official Gazette of the United States Patent and Trademark Office Patents, (Nov. 28, 2000) Vol. 1240, No. 4, pp. No Pagination. e-file.<br>ISSN: 0098-1133. |
| DOCUMENT TYPE: | Patent |
| LANGUAGE: | English |

AB An immunoassay for **Sarcocystis neurona** antibodies in **equines** is described. The immunoassay uses blocking of Sarcocystis antigens by antibodies to Sarcocystis sp.

other than **Sarcocystis neurona** in connection with the immunoassay.

L33 ANSWER 7 OF 9 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 2000152631 MEDLINE
DOCUMENT NUMBER: 20152631 PubMed ID: 10690772
TITLE: Improvement of western blot test specificity for detecting **equine** serum antibodies to **Sarcocystis neurona**.
AUTHOR: **Rossano M G**; **Mansfield L S**; Kaneene J B; **Murphy A J**; Brown C M; Schott H C 2nd; Fox J C
CORPORATE SOURCE: Animal Health Diagnostic Laboratory, The Population Medicine Center, Michigan State University, East Lansing 48824, USA.
SOURCE: JOURNAL OF VETERINARY DIAGNOSTIC INVESTIGATION, (2000 Jan) 12 (1) 28-32.
Journal code: A2D; 9011490. ISSN: 1040-6387.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200003
ENTRY DATE: Entered STN: 20000330
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Entered Medline: 20000321

AB **Equine** protozoal myeloencephalitis (EPM) is a neurological disease of **horses** and ponies caused by the apicomplexan protozoan parasite **Sarcocystis neurona**. The purposes of this study were to develop the most stringent criteria possible for a positive test result, to estimate the sensitivity and specificity of the EPM Western blot antibody test, and to assess the ability of bovine antibodies to Sarcocystis cruzi to act as a blocking agent to minimize false-positive results in the western blot test for **S. neurona**. **Sarcocystis neurona** merozoites harvested from **equine** dermal cell culture were heat denatured, and the proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in a 12-20% linear gradient gel. Separated proteins were electrophoretically transferred to polyvinylidene fluoride membranes and blocked in 1% bovine serum albumin and 0.5% Tween-Tris-buffered saline. Serum samples from 6 **horses** with **S. neurona** infections (confirmed by culture from neural tissue) and 57 **horses** without infections (**horses** from the Eastern Hemisphere, where **S. neurona** does not exist) were tested by Western blot. **Horses** from both groups had reactivity to the 62-, 30-, 16-, 13-, 11-, 10.5-, and 10-kD bands. Testing was repeated with another step. Blots were treated with bovine S. cruzi antibodies prior to loading the **equine** samples. After this modification of the Western blot test, positive infection status was significantly associated with reactivity to the 30- and 16-kD bands ($P<0.001$, Fisher's exact test). The S. cruzi antibody-blocked Western blot had a sample sensitivity of 100% and sample specificity of 98%. It is concluded that the specificity of the Western blot test is improved by blocking proteins not specific to **S. neurona** and using reactivity to the 30- and 16-kD bands as the criterion for a positive test.

L33 ANSWER 8 OF 9 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 2000043702 MEDLINE
DOCUMENT NUMBER: 20043702 PubMed ID: 10577742
TITLE: Simplified technique for isolation, excystation, and culture of Sarcocystis species from opossums.
AUTHOR: **Murphy A J; Mansfield L S**
CORPORATE SOURCE: Animal Health Diagnostic Laboratory, Michigan State University, East Lansing 48824, USA.
SOURCE: JOURNAL OF PARASITOLOGY, (1999 Oct) 85 (5) 979-81. Journal code: JL3; 7803124. ISSN: 0022-3395.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199912
ENTRY DATE: Entered STN: 20000113
Last Updated on STN: 20000113
Entered Medline: 19991202

AB **Sarcocystis neurona** is a protozoan parasite that causes a neurological disease in **horses** called **equine** protozoal myeloencephalitis. The route of transmission is speculated to be by fecal-oral transfer of sporocysts shed from opossums. Controversy exists regarding both the natural life cycle for this parasite as well as the species identity of opossum Sarcocystis. To provide stage-specific material for species comparison, 27 opossums from southern Michigan were screened for Sarcocystis spp. sporocysts. Seven opossums were positive for Sarcocystis sporocysts by fecal flotation. A simplified, effective technique for isolation, excystation, and culture of opossum Sarcocystis sp. from mucosal scrapings was developed. All 7 Sarcocystis sp. isolates were successfully cultured to grow long term in **equine** dermal cells to the merozoite stage. Merozoites were observed between 5 and 15 days after inoculation. In conclusion, opossums shed Sarcocystis sp. sporocysts that may be manipulated to excyst and grow in vitro in **equine** dermal cell lines to the merozoite stage using the simplified technique described.

L33 ANSWER 9 OF 9 CONFSCI COPYRIGHT 2001 CSA
ACCESSION NUMBER: 1999:13271 CONFSCI
DOCUMENT NUMBER: 99-025765
TITLE: Improved specificity of western blot detection of Sarcocystis neurona
AUTHOR: **Rossano, M.G.**; Mansfield, L.S.; Kaneene, J.B.; **Murphy, A.J.**; Brown, C.; Fox, C.J.
CORPORATE SOURCE: Michigan State Univ., East Lansing, MI, USA
SOURCE: Iowa State University Press (ISUP), 2121 South State Avenue, Ames, IA 50014-8300, USA; phone: (800) 862-6657; fax: (515) 292-3348; email: orderssupress.edu; URL: www.isupress.edu, Abstracts available. Contact ISUP for price. Paper No. 110. Meeting Info.: 984 5049: Research Workers in Animal Diseases (9845049). Chicago, IL (USA). 8-10 Nov 1998. Merial Limited, Origen, Pfizer, Fort Dodge Animal Health, Immtech Biologies, Pharmacia Upjohn, American Journal of Veterinarian Research, Elanco Animal Health, Grand Labs, Heska Corp..

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DOCUMENT TYPE: Conference
FILE SEGMENT: DCCP
LANGUAGE: English

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L35 0 S L34 NOT L32

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